UNIVERSIDAD COMPLUTENSE DE MADRID
FACULTAD DE PSICOLOGÍA

TESIS DOCTORAL

Brain networks in subjective cognitive decline and mild cognitive impairment: characterizing the predementia stages using magnetoencephalography

Redes cerebrales en quejas subjetivas de memoria y deterioro cognitivo leve: caracterización de las etapas de pre-demencia mediante magnetoencefalografía

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

PRESENTADA POR

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A mi familia: mi padre, mi madre y mi hermana. Porque aunque suene a tópico, sin vuestra ayuda y apoyo a lo largo de toda mi vida nunca habría podido escribir estas palabras. Gracias por haberme apoyado siempre y haberme dado la oportunidad de estudiar durante tantos años sin ninguna presión añadida. Gracias por haber estado a mi lado en los momentos más duros, cuando los caprichos de la vida decidieron que las cosas serían un poco más difíciles, por no haberme dejado caer en ningún momento de aquel aciago año.

Gracias por último, y más importante, a Silvia, A TI. Gracias por haber sido mi motor durante todos estos años, porque soportaste a mi lado cada adversidad e hiciste que cada buen momento, fuese aún mejor al compartirlo contigo. Gracias por darme estabilidad, por ayudarme a digerir y a no desmotivarme en cada noche de "ahora no sale nada". Por aceptar y compartir conmigo este camino que he decidido recorrer, a pesar de la inestabilidad y la incertidumbre, sin cuestionarlo jamás. Porque eres y serás parte fundamental de todo esto: antes, durante y después.

Acronyms

Aβ	Beta-amyloid
AD	Alzheimer´s Disease
aDMN	Anterior default mode network
AEC	Amplitude envelope correlation
APA	American Psychiatric Association
APP	Amyloid precursor protein
BEM	Boundary element method
BOLD	Blood Oxygenation Level Dependent
CAT	Computerized axial tomography
CO	Cingulo-opercular network
DAN	Dorsal attentional network
DCL	Deterioro Cognitivo Leve
DMN	Default mode network
DTI	Diffusion tensor imaging
ECoG	Electrocorticography
EEG	Electroencephalography
EKG	Electrocardiogram
EOG	Electrooculogram
FC	Functional connectivity
FDG	F-2-fluoro-2-deoxy-D-Glucose
fMRI	Functional magnetic resonance imaging
FPN	Fronto-parietal network
HC	Healthy control

HPI	Head position information
Hz	Hertz
ICA	Independent component analysis
iEEG	Intracranial electroencephalography
IWG	International Working Group
MCI	Mild cognitive impairment
MEG	Magnetoencephalography
MMSE	Mini Mental State Examination
MNI	Montreal neurological institute
MRI	Magnetic resonance imaging
NIA-AA	National institute on aging and the Alzheimer´s Association
PCC	Posterior cingulate cortex
pDMN	Posterior default mode network
PiB	Pittsburgh compound B
PLV	Phase locking value
QSM	Quejas Subjetivas de Memoria
RSN	Resting state network
SQUID	Superconducting quantum interference device
SSS	Signal Space Separation
T	Tesla

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Resumen

La demencia es un cuadro que puede ser originado por múltiples causas, produciendo un deterioro cognitivo muy marcado y limitando la independencia del paciente. La causa más común de demencia es la Enfermedad de Alzheimer (EA) que representa aproximadamente el 60% de los casos totales. Aunque existen numerosos factores que parecen modular el riesgo de desarrollar EA tales como factores genéticos (APOE, PS1, etc.) o variables relacionadas con el estilo de vida (estudios, ocupación, dieta, etc.), la edad es sin duda la variable más influyente y el mayor factor de riesgo ante la aparición de la EA. Por este motivo, el número de personas mayores afectadas por esta enfermedad no ha parado de aumentar durante las últimas décadas, y se espera que aumente su incidencia aún más.

Debido al fracaso generalizado de los ensayos farmacológicos, numerosos esfuerzos en investigación se centran ahora en la detección temprana de la EA. El curso de la EA es lento e insidioso, y la acumulación de neuropatología puede comenzar hasta 15 años antes de su diagnóstico. A lo largo de esta etapa preclínica los pacientes atraviesan un estadio conocido como deterioro cognitivo leve (DCL). Esta etapa se caracteriza por alteraciones en uno o varios dominios cognitivos que no genera aún graves alteraciones del funcionamiento diario. Este estadio está altamente asociado al desarrollo posterior de EA y por tanto se considera bajo determinadas condiciones una etapa prodrómica de la enfermedad. Las personas mayores con DCL suelen presentar alteraciones a nivel cerebral o metabólico característicos de la EA, tales como atrofia cortical, alteraciones sinápticas o acumulación de proteínas relacionadas con la fisiopatología de la EA.

La literatura científica reciente ha descrito una etapa anterior incluso al DCL que podría asociarse al desarrollo de demencia futuro. Las quejas subjetivas de memoria (QSM) se caracterizarían por la presencia de un sentimiento subjetivo de deterioro cognitivo en ausencia de afectación objetiva, es decir, la evaluación neuropsicológica de estas personas mayores se encuentra en el rango normal. Sin embargo, el estado de la actividad cerebral en esta etapa, o su integridad estructural aún no ha sido apenas descrito. Existen resultados contradictorios con respecto a si la presencia de QSM en personas mayores se asocia a un riesgo más elevado de desarrollar demencia. Además, mientras algunos estudios reportan alteraciones a nivel cerebral compatibles con EA en esta etapa, otros no encuentran tales signos.

El objetivo fundamental de esta tesis es la caracterización de las alteraciones en las redes cerebrales en personas mayores sanas, personas

mayores con QSM y personas mayores con DCL. El estado actual de la literatura nos permite anticipar la presencia de alteraciones cerebrales relacionadas con EA en el grupo con DCL, sin embargo este trabajo pretende estudiar si dichas alteraciones, o formas más sutiles, se encuentran presentes en el grupo con QSM. Esto nos permitirá en primer lugar clarificar si las QSM tienen alguna relevancia clínica y si se encuentran asociadas a cambios objetivos en la actividad cerebral. Además, se podrá describir el curso exacto de las alteraciones que tienen lugar a lo largo de las etapas preclínicas en la EA gracias a la inclusión del grupo con DCL, caracterizando así en cada estudio las dos etapas que anteceden a la EA descritas a día de hoy.

En el primer experimento se compararon los espectros de potencia de los tres grupos durante el estado de reposo. Tanto el grupo de QSM como el de DCL mostraron una reducción significativa de la potencia relativa en la banda alfa que afectaba a múltiples regiones cerebrales. Estos cambios se relacionaban con un peor rendimiento cognitivo. Además, únicamente el grupo DCL mostró un enlentecimiento significativo del pico alfa. Este enlentecimiento predecía una peor ejecución en múltiples escalas cognitivas. Por último, se comprobó que el volumen de hipocampo sólo estaba reducido en el grupo con DCL, mientras que el grupo QSM no mostraba cambio alguno.

En el segundo experimento se evaluaron los patrones de conectividad funcional en la banda alfa y se constató que tanto el grupo DCL como QSM mostraban un patrón claramente distinto al de los controles. Más concretamente, la sincronización entre regiones anteriores en ambos grupos era más intensa que en los controles, mientras que en regiones posteriores había claros signos de hipo-sincronización en los dos grupos preclínicos. Estas alteraciones en los patrones de conectividad funcional afectaron regiones muy similares en ambos grupos, relacionándose con un peor rendimiento cognitivo.

En el tercer estudio se estudió la organización de las redes cerebrales a lo largo de las etapas preclínicas de la EA. Se constataron alteraciones a nivel de red tanto en el grupo de DCL como en QSM, aunque en este último eran mucho más reducidas y limitadas a menos medidas topológicas. Además, el grupo con QSM mostró cambios en la modularidad y la transitividad así como en la partición modular de sus redes en la banda alfa en sentido opuesto a las alteraciones observadas en esos mismos parámetros por los participantes con DCL. Estos cambios fueron interpretados como posibles signos compensatorios que podrían subyacer el normal funcionamiento cognitivo en el grupo con QSM.

Los resultados de esta tesis permiten afirmar que las personas mayores con QSM muestran alteraciones a nivel sináptico significativas. Además, estas alteraciones son coherentes con las manifestadas por el grupo DCL, aunque de menor intensidad. Estos resultados permiten plantear un continuo a lo largo de las etapas preclínicas de la EA. En este continuo, las personas mayores atravesarían en primer lugar una etapa caracterizada por la presencia de preocupación subjetiva por su estado cognitivo, sin alteración significativa del mismo. En etapas posteriores la mayor acumulación de carga patológica conduce a un deterioro mensurable, que caracteriza la etapa de DCL.

Estos hallazgos suponen un relevante avance en la crucial tarea de descubrir biomarcadores tempranos de la EA. Dichos biomarcadores podrían permitir en un futuro la identificación de aquellas personas en riesgo de desarrollar EA para así administrarles tratamientos preventivos o programas de estimulación específicos.

Abstract

Dementia is a clinical entity producing major cognitive impairment that interferes with daily living activities that can be caused by a variety of conditions. Among them, Alzheimer´s Disease (AD) represents around a 60% of the total dementia cases. AD risk is modulated by multiple variables such as genotype (APOE, PS1, etc.) or lifestyle variables (studies, occupation, dietary patterns, etc.), although age is the most crucial risk factor for AD development. As a consequence, the number of AD patients has rapidly grown over the last few decades and is expected to increase even more dramatically in the near future.

Given the poor results obtained in pharmacological trials to cure or slow AD progression, early AD detection is receiving increasing research efforts over the last few years. Considering the slow and insidious progression of AD, brain pathology starts accumulating in the brain as soon as 15 years before clinical symptoms are severe enough to establish an AD diagnostic. Before reaching AD dementia, patients develop mild cognitive impairment (MCI). This stage is characterized by the presence of a significant cognitive impairment affecting one or more domains. However, this cognitive decline does not significantly limit patients' daily functioning. MCI patients are known to show increased conversion rates to AD with respect to healthy elders and thus this stage is commonly accepted as a prodromal stage of AD according to recent MCI criteria. MCI patients are known to exhibit AD-like brain and metabolic alterations such as cortical atrophy or AD-related protein accumulation.

Recent scientific literature has described a stage preceding MCI which could be associated with future dementia development. Subjective cognitive decline is defined by the presence of a subjective feeling of cognitive worsening in the absence of objective impairment in classical neuropsychological assessment. However, the integrity of brain activity or structure has been scarcely described yet. Furthermore, there exist some contradictory results regarding whether the presence of cognitive concerns is truly related to increased dementia risk. In the same vein, some studies have found brain alterations in SCD patients resembling of those associated with AD while others failed to find such signs.

The main objective of this thesis is characterizing brain network alterations in healthy elders, elders with SCD and elders with MCI. The current state-of-the-art lets us anticipate the presence of brain disruption in the MCI group, nonetheless, this work aims to provide evidence of whether similar alterations are already present in the SCD stage. The results presented in this thesis will clarify the clinical relevance of SCD by discerning whether

cognitive concerns are truly mediated by network disruption or not. Moreover, the exact course and development of electrophysiological brain alterations during the preclinical stages of the disease will be described by including also MCI patients. By including these three groups we will be able to characterize brain function in the different AD preclinical stages considered in current literature.

In the first experiment, spectral power distribution in the resting state was compared among the three groups. Both SCD and MCI groups showed a significant alpha relative power decrease affecting broad brain regions. These changes were associated with a worse cognitive status. Furthermore, only MCI patients showed alpha peak slowing. This slowing also predicted cognitive impairment in several cognitive domains. Lastly, hippocampal volume was only affected in the MCI group, while SCD showed preserved anatomical integrity in this region.

The second experiment focused on studying functional connectivity (FC) patterns in the alpha range. FC alterations were evident in MCI and SCD elders when compared to healthy controls. More concretely, both groups showed increased synchronization values over anterior brain regions while posterior areas exhibited hypo-synchronization. These alterations in the normal FC pattern affected widespread regions, but shared a common spatial pattern in both clinical groups. Furthermore, cognitive decline in our sample was related to FC disruption.

In the third experiment presented in this thesis brain network topology and organization throughout preclinical AD stages was assessed. Network disruptions in the MCI patients were found for several metrics, while SCD showed some signs of network disorganization but reduced to certain graph metrics. Interestingly, SCD showed changes in network modularity, transitivity and modular partitions in the alpha band that were interpreted as possible compensatory mechanisms, as they were opposed to those changes observed in MCI in each parameter. These changes could underlie cognitive maintenance in SCD elders.

The set of experiments detailed in this work lets us conclude that brain networks in the SCD stage are already significantly impaired. These functional alterations are coherent with those observed in MCI and follow a similar trajectory, although they are less severe in the former. These results as a whole allow drawing a continuum throughout the preclinical stages of the disease. In this continuum, elderly would first experience a subjective feeling of cognitive decline, while our current neuropsychological tools would not be sensitive enough to detect any objective impairment. In later stages subsequent brain pathological burden would lead to a significant cognitive decline associated with the MCI stage.

These findings represent a relevant step forward in the search for early AD biomarkers, which is a crucial challenge in modern science. Such biomarkers would allow the early identification of at-risk population, who would represent key targets for early pharmacological or behavioral interventions.

1. General introduction

1.1. Introduction to neuroimaging

Understanding our organism and behavior has represented historically one of the major challenges in human search for knowledge. In this travel, the brain represents a key to reach, as it underpins each of our acts, abilities or wills. As psychologists our main purpose is to understand human behavior. In particular, neuroscience seeks to unravel the biological mechanisms underlying our mental activity, i.e. the interactions between nervous system and behavior.

The first steps in this direction were mainly based on lesion studies (localized strokes, wounds, etc.) or direct electrical stimulation over brain surface. The idea of brain localizationism emerged from these studies, relating the loss or impairment of a certain cognitive function with a lesion in a given brain area (shown in Figure 1-1). There are some paradigmatic cases described in the literature such as Phineas Gage orbitofrontal lesion and his dramatic personality changes, "Tan" patient and his difficulties with spoken language caused by a left frontal lesion and the well-known patient H.M. suffering from anterograde amnesia after bilateral medial temporal lobectomy. All these cases illustrated how specific brain regions played an important role for particular tasks.

Figure 1-1. Schematic representation of functions localization in the brain in 1957 based on lesions and cortical stimulation. Reproduced from Polyak (1957)

During the 19th and 20th centuries there has been an exponential growth in the field of neuroscience as the number and variety of techniques to study brain behavior and morphology has experienced an unprecedented increase since its early days. The discovery of the microscope and staining

procedures laid the foundations of nervous system anatomical characterization, culminating in Santiago Ramón y Cajal´s (1843-1926) seminal work. He described for the first time the nervous system as an intricate ensemble of individual and autonomous cells, called neurons, which establish connections at specific locations among them (Ramón y Cajal, 1888). Ramón y Cajal, considered by many the father of modern neuroscience, was a scientist far ahead of his time who studied the changing nature of the nervous system, addressing topics still active nowadays in modern neuroscience such as brain development, neurodegenerative processes and neural regeneration. Some decades later, in the twentieth century and after World War II, a huge amount of advances arose in the fields of medicine and imaging in particular. The methods employed to image the brain up until then, such as angiography and pneumoencephalography were highly risky and extremely invasive for the patients (Mishra & Singh, 2010). One of the major contributions to modern neuroimaging was made by William H. Oldendorf (Oldendorf, 1961), who imagined, and eventually developed the first prototype of a modern Computerized Axial Tomography (CAT), which he termed as "Radiant Energy Apparatus".

Regarding electrophysiology, besides many important previous findings such as Galvani´s studies of the frog´s muscles contraction after death by applying an electrical current, the first recording of a human brain rhythm was carried out by Hans Berger (1873-1941) in the early twentieth century. He characterized an oscillatory activity ranging between 8 and 12 Hz appearing in a relaxed but alert state when eyes were closed. As it was the first human brain rhythm identified it was called "Alpha rhythm". Some years later, two more brain rhythms were identified by William Grey Walter (1910-1977): the theta rhythm (4-8 Hz) and the delta rhythm (2-4 Hz), which he used for the detection and localization of brain tumors. These discoveries have ultimately led to the development of electroencephalography (EEG) and magnetoencephalography (MEG). These techniques allow a much more sophisticated recording of the neural activity over a broad number of scalp positions as will be explained in more detail in forthcoming sections.

The following section contains a brief introduction and explanation about the basic features and information that can be obtained through some of the most representative structural and functional techniques. Obviously, a thorough review of the different techniques available as well as a meticulous explanation about each of them is out of the scope of this thesis.

Nowadays, a variety of techniques are available that allow us to characterize and investigate either brain structure or brain functioning, or even both of them at a time. As one could expect, different neuroimaging

modalities will provide us with complementary information about brain functioning or structure. It is crucial to bear in mind what question about the nervous system the research is trying to solve. Some techniques offer a high precision pointing out sharp structural details and distinguishing different activations in small and close regions with a great exactitude. This is called spatial resolution. The flip side of the coin is temporal resolution. A neuroimaging technique capable of distinguishing different brain events occurring in a short fraction of time has high temporal resolution. In an ideal scenario a combination of high temporal and spatial resolution would provide comprehensive information about both brain dynamic and structure. In practice, imaging modalities are characterized by a given temporal and spatial resolution, and often the decision of what modality is more appropriate for a given study implies a tradeoff between both characteristics.

1.1.1. Structural imaging techniques

Structural techniques are useful to visualize the anatomy and structure of the nervous system. They can be used in a variety of ways in brain research and in the clinical setting, and their results can be linked to cognitive processes or degenerative diseases. Each structural technique inspects a different aspect of the nervous system, for example: distinguishing between different types of tissues such as cerebrospinal fluid, gray matter or white matter, measuring the volume or thickness of specific brain regions related to the cognitive process of interest, or trace the white matter fiber tracts connecting different brain regions amongst other uses. These are some of the most common techniques employed to study brain structure:

Computerized axial tomography

One of the earliest techniques to determine brain structure was Computerized Axial Tomography (CAT). Even though CAT is still largely used, it has been partially replaced by MRI, which is more powerful and provides more detailed information (Figure 1-2), as explained in following sections. CAT is based on X-ray imaging. When using conventional X-rays a single 2-dimensional image of the whole volume is taken with the consequent loss of depth information, as all the structures overlap in a single photograph. For instance, when trying to localize a carcinoma in a front-to-back chest X-ray image, the radiologist would not be able to determine how back the carcinoma is localized in the lung. CAT solves this problem by reconstructing multiple X-ray images, each of them representing a slice of the whole 3-dimensional image (Orrison, Lewine, Sanders, & Hartshorne, 2017). A thin collimated beam of X-rays passes through the body repeatedly through different angles reaching the sensor. The sensor measures how the X-rays have been attenuated while passing the body. Finally a 2D image can be reconstructed in any desired angle in which each

pixel represents the amount of radiation absorbed in that specific space location. CAT is a non-invasive technique although the subject is exposed to a low amount of radiation coming from X-rays. Because of its technical properties it is especially suited for the visualization of high density tissues, such as bones, and not as good for low density organs. CAT offers a quite good spatial resolution, under 1 mm. Among its clinical uses, cancer diagnosis and follow-up is one of the most common, although it is also employed in neuroimaging to evaluate traumatic head injury or vertebral fractures.

Magnetic resonance imaging

Magnetic resonance imaging (MRI) is a largely used technique to produce high quality images of the different body tissues based on the principles of magnetic nuclear resonance. In particular, MRI scanners generate strong magnetic fields which passing through the body, altering hydrogen atoms, which ultimately emit a radio frequency signal that is captured by sensors in the scanner. These radio frequency signals reflect some properties of the tissue in which they are placed (Orrison et al., 2017).

MRI signals are generated mainly by hydrogen nuclei. The human body is mainly formed by fat and water, which are in turn rich in hydrogens atoms. The nucleus of a hydrogen atom has only one proton with a particular rotation, or spin, so that they can be roughly conceived as small magnets. There are around 1025 hydrogen atoms in a single gram of water, each of them with its own random spin orientation. An MRI scanner produces enormous magnetic fields that alter the random orientation of these hydrogen protons, aligning them with the orientation of the field. In fact, due to the movements and nuclear interactions between all the different atoms in the tissue there is only a small tendency for the spins to point along the field. The spins of hydrogen protons are still largely pointing in random directions even in the presence of the strong magnetic field produced by MR scanner. However, this small tendency to align in a particular direction is strong enough to produce a net spin direction when summing over the many hydrogen atoms present in any small piece of tissue. MR scanners typically use magnetic field of around 0.5 to 7 Tesla (T), with bigger magnetic fields producing greater alignment and thus increasing signal-to-noise ratio. The alignment movement is accompanied by a precession movement rotating around the direction of the static magnetic field applied by the scanner. This precession movement has a particular frequency depending on the intensity of the field, called the Larmor frequency. After hydrogen atoms are aligned with the static magnetic field, a pulse of electromagnetic waves at the above mentioned frequency (in the radio frequency range) is applied orthogonally with respect to the strong magnetic field. Once the radio frequency wave pulse ends, the protons tend

to go back to their previous state by releasing energy in the form of a radio signal at the precessing frequency, the so-called free induction decay measured by receiver coils in the MRI system (Maestú, Rios Lago, & Alonso, 2008).

By measuring the relaxation time of the protons in the longitudinal axis (T1), or in the transverse axis (T2) we can appreciate different aspects of body tissues: this is called image weighting. Different combination of scanner parameters can modify the specific sensitivity to different tissues by increasing the sensitivity to T1-related changes (with high contrast between white matter, gray matter and cerebrospinal fluid, allowing the detection of morphology-related pathologies) or T2-related changes (especially suited for detecting soft tissue pathologies).

MRI is a highly used technique in the clinical setting in oncology, soft tissue damage and especially in neurology and neuroscience. A brain MR images can be segmented into the different tissues (gray matter, white matter, etc.) and even the volume of individual structures can be calculated from each individual image to compare structure size across subjects or group of subjects. It also offers an excellent spatial resolution which is physically limited to 10 μm because of water movement during acquisition. However, achieving this resolution would need an extremely long acquisition time, thus resolutions around 1 mm are more common in research and clinical practice

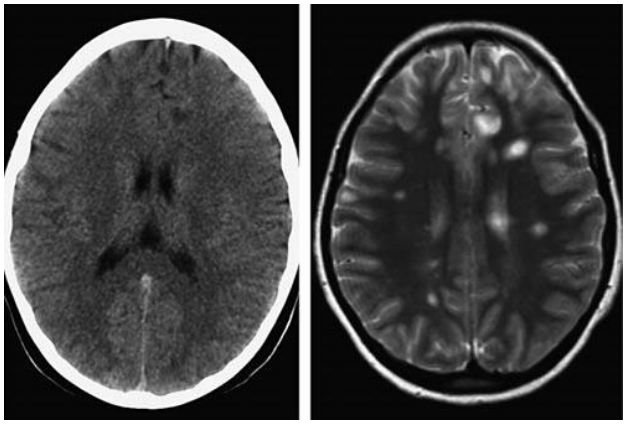

Figure 1-2. White matter lesions are very difficult to detect in CT (left) compared to MRI (right). Reproduced from Barkhof & Scheltens (2002).

Diffusion tensor imaging

MR allows for great variety of contrast mechanisms which makes MRI a very flexible tool to study the nervous system. A specific configuration in the acquisition sequence enables MRI to detect the microscopic random

movements of water molecules. This technique was first introduced in the mid-90s (Basser, Mattiello, & LeBihan, 1994) to reveal molecular mobility which can be interpreted in terms of microstructure and anatomy information at each voxel. Diffusion tensor imaging (DTI) makes use of the so-called Brownian motion which is a random thermal motion as a probe to infer neuroanatomy. The information DTI provides is dominated by anatomy, providing no information about neurophysiological processes. DTI measures the spreading of water molecules into brain tissue. If water molecules are able to move freely and equally in all directions, then there is maximal isotropy. On the other hand, if water molecules move only over one direction then there is maximal anisotropy. DTI makes use of this anisotropy metric to infer axonal organization in the brain. White matter fibers limit the ability of water molecules to spread in all directions and show preferential diffusion over the axon direction (Figure 1-3). However, if fiber tracts are not firm and compact enough or if there is a break in these tracts, then the anisotropy of water diffusion decreases, providing us with information about brain axonal structure (Le Bihan et al., 2001; Maestú et al., 2008).

Although DTI is not a really extended technique in the clinical settings, it has raised high interest and expectations for its promising results in neuroinmonology (e.g., multiple sclerosis) and traumatic brain injury, as it enables the detection of diffuse axonal injury which cannot be detected with regular MRI. Furthermore, it is very useful in research for structural connectivity estimation. Once tracts have been reconstructed following the maximal diffusivity direction of water molecules, a structural connectivity metric can be calculated through the number of fibers connecting two regions or the average fractional anisotropy of this tract. These technique have contributed to improving our understanding of brain development and functioning (Dennis & Thompson, 2014).

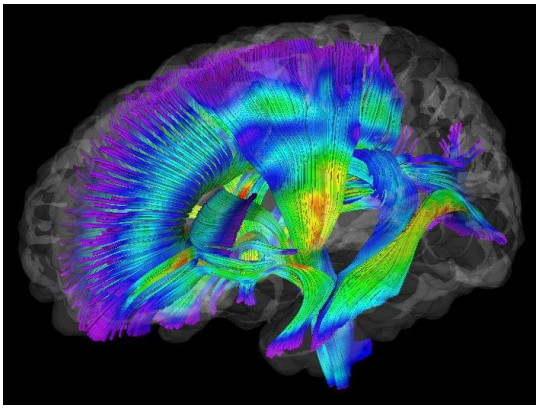

Figure 1-3. Tractography reconstruction from DTI showing white matter fibers.

1.1.2. Functional imaging techniques

While structural techniques map the shape and organization of the nervous system itself, functional techniques provide the possibility to *observe* brain functioning and how different brain regions become active in order to solve a problem or perform a task. From the psychological perspective functional techniques are of extreme interest. Even though certain structural measures, such as the volume of specific brain regions or white matter connection between brain areas, can shed light into some cognitive processes, functional techniques enable us to directly measure how our experimental manipulations change brain functioning. Not so long ago, scientists had to wait until death to study the brain of their patient, while psychologists could only rely on very specific cases of localized lesions to study the relationship between brain and behavior. The raise of modern functional neuroimaging enabled psychologists to start unraveling the relationship between brain and emotion, to investigate with split-second precision the neural processes underlying memory or to assess the effectiveness of certain treatments. In this section the following functional imaging techniques will be introduced: Positron emission tomography (PET), functional magnetic resonance imaging (fMRI), and electrophysiological techniques such as magnetoencephalography (MEG) and electroencephalography (EEG). The latter group, and more concretely MEG, will be covered in more detail in following sections within the methods employed for the development of the three experiments of this thesis.

Positron emission tomography (PET)

PET is included in this section as a functional technique it can image both structure and function (Ter-Pogossian, 1983). However, one of its most common uses in neuroimaging and in common clinical practice is to assess brain or whole body metabolism. Although the idea of *in vivo* measurement of biological and biochemical processes was already present in the 1930s it did not become reality until past 1980, when the first PET scan became available. PET scan functioning is based on the detection of the electromagnetic radiation derived from the decay of a certain radiopharmaceutical. More concretely, these radionuclides are made of instable molecules specifically designed to trace or mark specific cells or other molecules to infer metabolism or other relevant clinical aspects. The radionuclides have a deficit of neutrons in their nucleus and become more stable by means of the nuclear transformation of a proton into a neutron. This process emits a neutrino and a positron, resulting in the eventual annihilation of the latter with an electron. This collision results in the liberation of two photons with the same trajectory but opposite direction which are measured in coincidence. Through advanced algorithms and imaging techniques, the regions where the radionuclides are more likely to decay are reconstructed, and thus it becomes possible to infer particular

aspects of metabolic functioning (Maestú et al., 2008). PET has clinical and research applications mainly in cardiology, oncology and neurology (Phelps, 2000). One of the most used radiopharmaceuticals is FDG (F-2-fluoro-2-deoxy-D-Glucose) which is a glucose analogue enabling the measurement of the glucose consumption *in vivo*. In neuroscience FDG-PET has been largely used to study which brain regions show higher metabolic rates, and thus are more active, for example, while executing a certain cognitive task. This radionuclide allows PET to unravel which brain regions are more active in certain cognitive processes as those regions should show higher FDG uptake associated with higher glucose consumption. It is also useful tool to asses Alzheimer's Disease (AD), as a significant reduction of metabolism has been reported for certain brain areas such as medial temporal regions in AD patients (Lehmann et al., 2014). Another relevant radiotracer for AD is Pittsburgh compound B (PiB). It is known to aggregate to amyloid depositions thus resulting useful to image β-amyloid plaques. This procedure allows tracking the progression of the amyloid pathology in the different stages and improves the accuracy of the diagnosis, which otherwise would rely on more invasive techniques such as a lumbar puncture. PET scan offers a spatial resolution of around 4 to 7 mm and is often combined with a structural CAT scan. However, the temporal resolution of PET is quite poor: in the order of tens of second or even minutes.

Functional magnetic resonance imaging

fMRI is a largely used neuroimaging technique that employs a regular MRI scanner to detect brain activation (Amaro & Barker, 2006). The discovery that MRI could be sensitive to brain activation was made by the fact that MR signal could increase in areas where intense brain activation was expected. Despite the fact that signal increase is quite small (around 1%), it is the basis of fMRI studies. Although quite complex, brain functioning relies on neuronal electrochemical transmission. All the electrical currents flowing through the neurons allow the exchange of neurotransmitters. However, all these processes require a high amount of energy and oxygen, in fact, brain activity consumes up to a 15% of the total energy that an individual needs per day (Martí-Climent, Prieto, López Lafuente, & Arbizu, 2010). Furthermore, the brain is a highly aerobic organ that is extremely dependent on oxygen supply to effectively fulfil its role.

fMRI is an indirect measure of brain activation. It does not capture the neuronal activity per se but rather the imprint it leaves behind. When a brain region is involved in a certain task its neurons become active and thus use a higher amount of energy and oxygen. This triggers an increase in the blood flow towards that area, which in turn increases the level of available oxygen surrounding that specific brain region. The most common fMRI

approach uses the Blood Oxygenation Level Dependent (BOLD) contrast. BOLD contrast uses the fact that hemoglobin exists in two forms to guarantee oxygen supply to the body: oxyhemoglobin (carrying oxygen molecules) and deoxyhemoglobin (without oxygen molecules), each with different magnetic properties (Pauling & Coryell, 1936). fMRI takes advantage of the different T2* properties of between oxygenated and deoxygenated hemoglobin. Because deoxygenated hemoglobin is paramagnetic, it suppresses T2*-weighted MR signal. Contrarily, an increase in oxygenated hemoglobin is associated with an increase in this same signal. When a region becomes active, and before there is additional blood supply in the area, neurons start consuming more oxygen, so the levels of deoxygenated hemoglobin rise which create a negative response in the BOLD signal in the first 1 or 2 seconds after activation (Menon et al., 1995), *the negative dip*. After that the blood flow is strengthened and the levels of oxyhemoglobin, and consequently BOLD response, both rise up. This BOLD response peaks at around 4 to 6 seconds after the stimulus onset, to then slowly decay and become stable around 20 seconds after a *post-stimulus undershoot* below baseline level (Lindquist, 2008). These changes in the MRI signal produced by neuronal activity are called the hemodynamic response function (see Figure 1-4).

fMRI offers a good spatial resolution, up to 1 mm, but ranging in the order of 3 mm usually. However, it does not excel in temporal resolution. Instead, it tracks the change in BOLD hemodynamic response in each brain voxel from one image to the next scan acquired. A single image acquisition takes seconds (usually 2 sec), thus limiting the temporal resolution of fMRI.

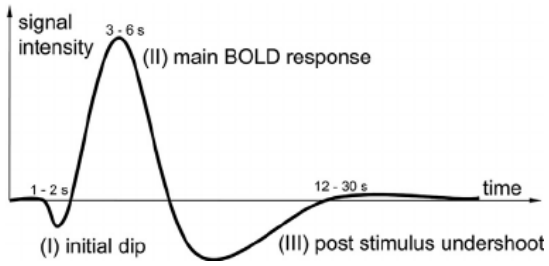

Figure 1-4. Schematic course of the hemodynamic response function typically found in fMRI experiments. Reproduced from Siero, Bhogal, & Jansma, (2013).

Electrophysiological techniques

The basis of modern electrophysiology started to settle two centuries ago, when Galvani introduced for the first time the idea of bioelectricity. He referred to it as “animal electricity” when he discovered by chance while dissecting a frog that its muscles produced electricity to move. Since then, scientific work has led to the understanding of how neurons and other cells employ electricity to communicate or to contract different muscles, and

how applying our current understanding about electromagnetic fields, we can measure that activity to study brain processes.

Electrical activity of the brain results from ionic fluxes generated by biochemical processes in the neurons. The principal generators of electrophysiological recordings are pyramidal neurons located in the brain cortex. Electroencephalography (EEG) uses a set of electrodes placed in the surface of the scalp which measure electric potential generated by these neuronal currents. However, the tiny neuronal currents can only produce a measurable signal at the scalp if the individual currents are aligned in the same direction. This phenomenon is called spatial summation. Pyramidal neurons in the cortex are parallel to each other giving rise to open field, where the electrical currents are spatially summated and can be recorded. On the contrary, when neurons are placed symmetrically, they create a closed field that cancels opposing electrical currents thus leading to a null overall electromagnetic field (Buzsáki, Anastassiou, & Koch, 2012). Additionally, temporal summation is also critical for EEG, the synchronous activation of a large number of neurons is needed to detect any signal over the scalp surface (Maestú et al., 2008).

Magnetoencephalography (MEG) is one of the most recent neuroimaging techniques: its invention took place only around 50 years ago. It is based in similar principles than EEG and it also relies on temporal and spatial summation of postsynaptic potentials (either excitatory or inhibitory) to record brain activity. However, MEG measures the tiny magnetic fields derived from pyramidal cell dendrites. MEG mainly detects activity originated in the brain sulci given its spatial distribution properties. While all the electrophysiological techniques show a great temporal resolution (in the order of less than 1 ms), its spatial resolution suffers when activity is recorded outside the scalp (i.e. EEG and MEG) and range from 1 to several centimeters, being significantly better in the latter.

MEG and EEG are both completely non-invasive techniques showing excellent temporal resolution; however, its spatial resolution can be improved when there are specific clinical demands to track neuronal activity with both excellent temporal and spatial precision. Electrocorticography (ECoG) is an invasive technique really popular in the clinical setting to study epilepsy specially. Subdural electrodes are placed directly over the surface of the cortex. Nevertheless, because of its extreme invasiveness (it requires a craniotomy to place the electrodes) it is limited to only few cases in the clinical setting. Its spatial resolution of around 5 mm (Buzsáki et al., 2012) can still be improved with a more invasive technique typically known as local field potential or intracranial electroencephalography (iEEG). iEEG makes use of thin metal or glass electrodes inserted directly into the brain tissue to obtain the electric activity

from the specific layer or structure of interest. Depending on the thickness of the electrode it can isolate and measure the activity of small neural patches or even single neurons. Figure 1-5 presents a schematic representation of the temporal and spatial resolution of the different functional techniques.

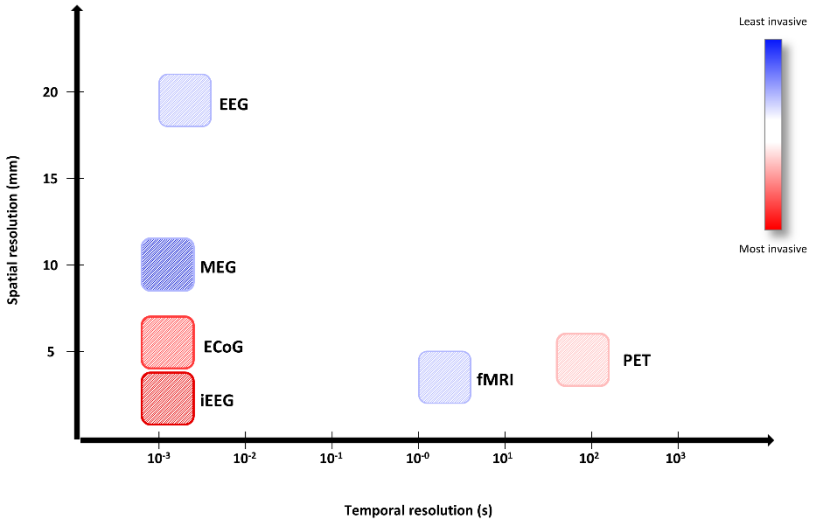

Figure 1-5. Temporal and spatial resolutions of the different neuroimaging functional techniques. Color code indicates invasiveness of the technique red invasive, blue non-invasive.

1.1.3. Brain oscillations and resting state

Brain rhythms characterization and physiology

Human behavior represents one of the most intriguing fields of study in modern science given its overwhelming complexity. Accordingly, psychologists have long been trying to fragment behavior into smaller components with a functional meaning, either in the radical behaviorism era when human functioning was reduced to the measurable relationships between stimuli and responses or in the later Gestalt school where perception of the world was the matter of study. In spite of the fact that these approaches resulted excessively simplistic to account for all the complexity in human behavior they definitely represented a step forward in our understanding.

A more recent approach in trying to understand this matter is the study and characterization of brain activity. Brain response as measured by MEG (or EEG) lays on an intricate pattern of electrochemical signaling between

neurons, resulting in an electromagnetic signal measurable from the outside of the scalp. This electrophysiological signal, however, presents an extraordinarily complex pattern difficult to address in psychological terms on its own. Electrophysiological activity can be also divided into different oscillations (or brain rhythms) which represent the fundamental organizer of neural information processing giving raise to cognitive processes (Buzsáki, 2006). An oscillation is a periodic movement (or change in a certain magnitude) around an equilibrium point that repeats itself in a regular cycle. Oscillations can be defined using only three parameters: the period (or its inverse, the frequency), the amplitude and the phase (Figure 1-6).

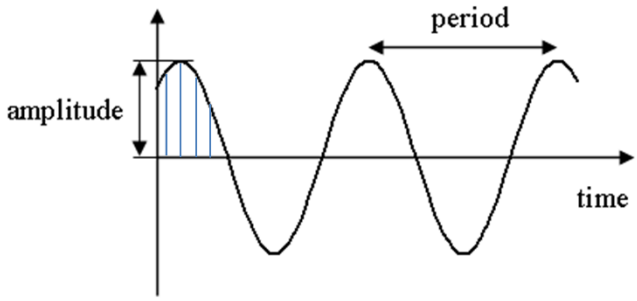

Figure 1-6. Schematic representation of an oscillation and its fundamental properties.

- Period: This is the time the oscillator takes to complete a cycle, *i.e.* to do a round trip from one point and return to the original position. It is measured in seconds. Additionally, the frequency of an oscillation reflects the number of cycles the oscillator completes in a second, and it is measured in hertz (Hz). Frequency is the inverse of the period (one divided by the period). The term frequency will be used from now on.

- Amplitude: It represents the maximum displacement of the oscillator from the equilibrium point.

- Phase: Is the exact position of an arbitrary point in the oscillation in a given time point.

As already mentioned, the alpha rhythm was the first spontaneous oscillation recorded from EEG scalp in human eyes-closed resting state. Furthermore, the study of eyes-open state led to the discovery of alpha-blockade, which in turn allowed the recognition of slower amplitude oscillations with a higher frequency, the beta waves. Around a decade later,

in the 40s, a set of high-frequency oscillations were discovered in the hedgehog related to smelling process, they were termed gamma band oscillations. Interestingly, most of the studies in the following decade were focused in this brain rhythm. Since the discovery of sensory evoked potentials in the 50s the enthusiasm about oscillations was dramatically dampened and neurophysiology was mainly focused on the characterization of new components in the evoked responses such as the well-known P300 component (Sutton, Braren, Zubin, & John, 1965) among many others. The interest in brain oscillations started growing in the 80s again and they became a useful analytical tool for assessing cognitive processes in the brain.

The brain adapts to a changing environment by modifying its oscillations in a variety of ways. This modifications can be directly linked and depend only on the characteristics and nature of the stimulus (evoked responses) or may also depend on intermediate cognitive processes (event-related or induced responses) (Karakaş & Barry, 2017). Current research put up a common feature to all brain rhythms. They are responsible for controlling when, and which kind of information perceived from the outside world by the peripheral sensors, can be distributed from the thalamus to the corresponding cortical areas to be processed and which one would be ignored (Buzsáki, 2006). However, identifying the specific functions of each rhythm is a really complex and controversial field and it remains largely unknown. This is partially due to the multiplicity in their relationship, that is to say, a single brain rhythm has been related to multiple processes -*e.g.* alpha activity has been linked to sensory processes (Başar, 1998) as well as motor regulation (Pineda, 2005)-, while a single cognitive process can be held by various brain rhythms at a time. According to the parallel distributed processing model, the brain would deal with the information by combining a large number of processing elements, enabling the processing of a greater amount of information. Başar (2011), proposed that the critical elements in sensory-processing and cognition could be specific patterns of oscillatory superpositions.

Even though the exact physiological basis of brain rhythms remain unclear, a number of studies suggest that thalamo-cortical loops play a crucial role in its generation (Hughes & Crunelli, 2005; Lőrincz, Kékesi, Juhász, Crunelli, & Hughes, 2009). Apparently, oscillations represent an efficient and optimus solution for large-scale prolonged isolation of the sensory inputs while they are not to be attended. The exclusive use of cortico-cortical connections would result in an increasing axon-conduction delay given the distance between cortical regions. Contrarily, the use of transthalamic fibers as a relay allow for a much more efficient interconnection between distant cortical regions. Furthermore, oscillations provide an optimal solution given the resonant properties of the nucleus

involved in oscillation generation, which amplifies the otherwise weak modular connections (Buzsáki, 2006).

Traditionally, electrophysiological activity has been classified into five bands with somewhat arbitrary limits: delta (0.5-4 Hz), theta (4-8 Hz), alpha (8-12 Hz), beta (12-30 Hz) and gamma (30-100 Hz). Additionally, ultra-slow waves (<0.2 Hz) and high frequency oscillations (>100 Hz) have been also studied to some extent (see Figure 1-7 for a schematic representation of band-pass filtering).

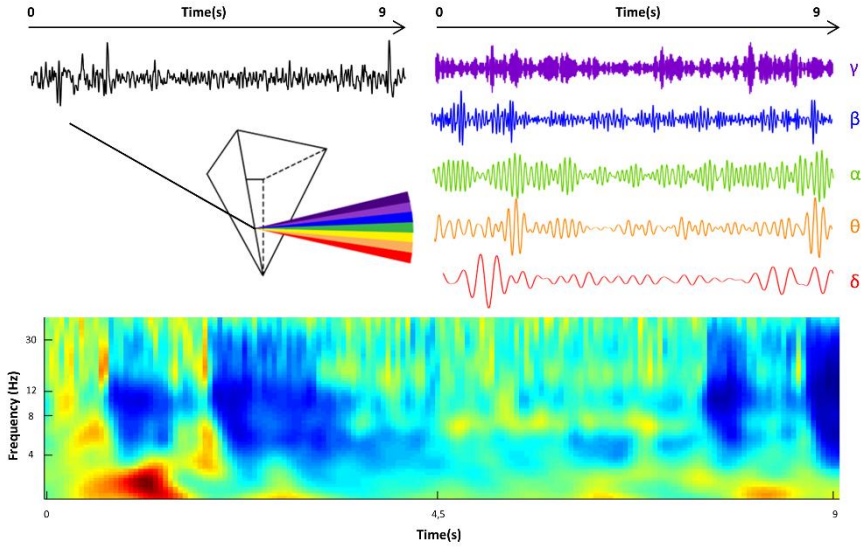

Figure 1-7. Schematic band-pass filtering process. A broadband signal (black line left-top side) is filtered into classical bands (color lines left-right side). The bottom part shows a time-frequency analysis of the same broadband signal; warm colors indicate power increases and cold colors indicate power decreases in a specific frequency (Y axis), at a specific time point (X axis)

- Delta band: Delta activity has been traditionally related to childhood and development. In fact, maturation was shown to be associated with a decrease in average peak delta amplitude and with a slowing of delta frequencies (Smith, Karacan, & Yang, 1977). Delta oscillations are tightly related to certain sleep phases, such as the slow wave sleep, to anesthesia (Amzica & Steriade, 1998).They are even used as an index of anesthesia depth (Billard, Gambus, Chamoun, Stanski, & Shafer, 1997). Delta activity is also present in wakeful state. However, spontaneous power increases in this specific band have been traditionally interpreted as a marker of pathology. Cortex regions adjacent to brain tumors and edematous areas showed more delta activity in a recent study (Oshino et al., 2007). Furthermore those patients showing more widespread delta activity

exhibited a poor recovery after surgery. Additionally, increases in delta band activity have been observed in patients with brain damage, more specifically in aphasic patients (Spironelli & Angrilli, 2009). Nevertheless, it is noteworthy that delta activity is not only present, but crucial, in normal brain processing and it has been found to be associated with a variety of cognitive processes. Delta band activity seems to play an important role when large-scale distant cortical regions coordinate their activity during decision-making tasks (Nácher, Ledberg, Deco, & Romo, 2013). These oscillations are also necessary for the regulation of basic autonomic functions and motivational processes such as defense or reward (Gennady G. Knyazev, 2012) and in attentional processing related to the detection of salient stimuli in the environment (G.G. Knyazev, Slobodskoj-Plusnin, & Bocharov, 2009). Lastly, sustained delta band activity exerts inhibitory control over regions that should be inactive during a task performance to avoid external interference (Harmony, 2013).

- Theta band: Theta band power similarly to delta activity is reduced along the maturational process in the normal child (Clarke, Barry, McCarthy, & Selikowitz, 2001). Afterwards, its relative power is also increased in normal aging (Clarke et al., 2001), thus following a similar maturational trajectory to delta activity. Furthermore, these increases are further accentuated in pathological conditions such as AD (Breslau, Starr, Sicotte, Higa, & Buchsbaum, 1989), or brain tumors (Oshino et al., 2007).

Besides alterations in theta activity linked to neurological conditions such as the above-mentioned; theta band represent a crucial brain rhythm for cognitive processes that has been largely studied, especially in rodents and primates. Theta activity generation is closely related to hippocampal formation. A special type of hippocampal neurons, called place cells seems to be implicated in orientation processes as well as planning navigation and movement (Sanders, Rennó-Costa, Idiart, & Lisman, 2015). This same work proposed that theta rhythm may serve different roles and functions along one cycle. More concretely, it is thought that while the first half of a theta cycle is devoted to processing and calculating the current position, the second half is in charge of planning and calculating the 'mind-travel' for the next few steps to be done with respect to the present position. In this same direction, it was proposed that theta activity was responsible for providing motor systems with updated sensory feedback of their performance with respect to the environment (Bland & Oddie, 2001).

Theta band has been also linked with active processing in high-level cognition, such as memory processes (Jacobs, Hwang, Curran, & Kahana, 2006), working memory retention (Raghavachari et al., 2006), attention (Cavanagh, Zambrano-Vazquez, & Allen, 2012) and monitoring of top-down executive control (Cavanagh & Frank, 2014). Human ability to

understand spoken language has been said to depend on theta activity. Particularly, motor cortex activity when trying to identify motor patterns in spoken language seems to rely on theta oscillations (Onojima, Kitajo, & Mizuhara, 2017). Furthermore, the specific frequency of theta oscillations can be modified to track syllable counting and identifying their boundaries to help the interpretation of spoken language. Finally, a direct link between theta activation and cognitive effort has been proposed, as theta oscillations track the increases in task demands (Gazzaley et al., 2008; Klimesch, 1999)

- Alpha band: Alpha activity, ranging between 8 and 12 Hz, represents the major component of the resting state activity in the human brain, and can be easily identified as the most prominent peak in the power spectrum. From an evolutionary perspective, the intensity and presence of alpha activity increases during childhood and adolescence (Jennekens-Schinkel, 1987) and then starts to decrease with ageing (Somsen, van't Klooster, van der Molen, van Leeuwen, & Licht, 1997). It has been demonstrated that the maturation of the posterior alpha rhythm is finished by the age of 16 (Marcuse et al., 2008). This changes in the main frequency of the alpha rhythm are of a high interest for neuroscience and psychology in particular given that they explain individual differences in processing speed and memory performance (Surwillo, 1971).

Alpha band oscillations are nowadays vastly studied and considered one of the main functional components of the sensory and cognitive processing in the brain. However, for entire decades it was considered an epiphenomenal of an idling brain, and thus not relevant for cognitive processing (Pfurtscheller, Stancák, & Neuper, 1996), or even brain noise (Karakaş & Barry, 2017). This misconception of alpha role was gradually changing. Busch & Herrmann (2003) demonstrated that alpha activity increased during a short term memory task in relation to the number of features and task difficulty. Furthermore, in a posterior study (Herrmann, Senkowski, & Röttger, 2004), alpha activation was shown to be greater during a retention interval in a memory task as compared to a purely perception condition, thus reflecting cognitive processing.

An increasing number of studies have demonstrated that the role of alpha band is far beyond a simple trace of an idling brain. Alpha modulations have been traditionally associated with top-down processes like working memory tasks (Pinal, Zurrón, Díaz, & Sauseng, 2015), cognitive control to avoid external interference (Bonnefond & Jensen, 2012) or selective attention (Banerjee, Snyder, Molholm, & Foxe, 2011). It has been proposed that event-related alpha synchronization exert inhibitory top-down control over certain cortical regions for cognitive control while its desynchronization reflects a decrease in this inhibitory control thus releasing cortical activation.

-Beta band: Berger first described beta waves as superimposed over the alpha rhythm. However, they were considered as muscular artifacts by many authors in the following years. It was only in the late 1930s when Jaspers and Andrews (1938) discovered an independent cortical origin for beta waves over sensorimotor regions. Although it has been typically associated with motor planning and execution (Pfurtscheller & Lopes da Silva, 1999), it has been studied less often than the above mentioned brain rhythms.

A similar line of evidence suggests that the feedback coming from sensory afferents is integrated to control static motor performance through increases in beta bursts activity (Lalo et al., 2007). Additionally, beta activity is also implied in cognitive tasks requiring motor interaction (Kilavik, Zaepffel, Brovelli, MacKay, & Riehle, 2013). Contrarily, some authors posit that the fact that motor control is very hard to disentangle from attentional processes is often overlooked, and thus, all the above-mentioned beta modulations regarding motor tasks are in turn accompanied by a variety of attentional processes. There is an increasing number of evidences supporting this interpretation (Gola, Magnuski, Szumska, & Wróbel, 2013; Wróbel, 2014). Besides, beta desynchronization in the inferior frontal gyrus has been related to a better performance in a word recall task (Hanslmayr, Matuschek, & Fellner, 2014), and even top-down control seems to depend on large-scale networks synchrony oscillating in beta frequency range (Bressler & Richter, 2015).

- Gamma band: Gamma activity was recorded for the first time as 50 Hz oscillations in the previously mentioned work by Jaspers and Andrews (1938), where beta band activity was also discovered. It was only in the late 1980s when scientific studies started to characterize the relevance of gamma band in cognition. The first few works in this regard pointed out the role of gamma in visual perception, particularly in binding together the different features of a stimulus for object representation (Gray, König, Engel, & Singer, 1989). Besides binding functions, which have gathered most of the attention, gamma band is also involved in attentional processes. For instance, attended stimuli promoted greater gamma activity than those not attended (Tiitinen et al., 1993) and improved synchronization in the gamma band in visual areas (Womelsdorf, Fries, Mitra, & Desimone, 2006) which increased the reaction times for detecting changes in attended stimuli (Hoogenboom, Schoffelen, Oostenveld, & Fries, 2010). The overall alertness state and the arousal of a subject seems to rely in gamma oscillations (Strüber, Basar-Eroglu, Hoff, & Stadler, 2000) as well as language perception (Eulitz et al., 1996).

Gamma oscillations are involved in bottom-up processes such as feature detection in visual perception modulated by to low-level characteristics of

the stimulus, like its size, eccentricity, etc. (Niko A Busch, Debener, Kranczioch, Engel, & Herrmann, 2004). In addition, top-down control is also mediated by gamma activity in working memory tasks comparing each new stimulus with previous ones, in such a way that matching stimuli enhance the gamma oscillatory response. These apparent discrepancies between the different roles of gamma band were reconciled with the Match and Utilization Model proposing that the early gamma component was devoted to feature binding and comparison with working memory, afterwards the late gamma component is in charge of the utilization of the new processed information, whether it should be stored or used to guide performance (Herrmann, Munk, & Engel, 2004).

Oscillations beyond gamma range, also known as high frequency oscillation, although less studied, are highly associated with epileptogenicity as they have been found to be specifically associated with cortical regions inducing seizures and are sensitive to predict its incoming appearance (Kobayashi et al., 2017). Nevertheless, even though these fast oscillations are sometimes pathological predictors, they can also play a physiological role, thus distinguishing between both types of activity is crucial.

Resting state significance as a cognitive and clinical biomarker

The study of resting state with neuroimaging implies the characterization of the different pattern of activation and interactions among regions in the absence of an explicit external task. Brain fluctuations in this context typically reflect the baseline activity when there is no goal-directed behavior. However, regardless of whether human brain is carrying out a particular task or not, brain activity exhibits certain spatio-temporal dynamics (Papo, 2013). These dynamical properties in resting state are not a mere reflection of a relaxed and inactive brain. On the contrary, they are influenced by previous inputs and also modulate future responses (Ohl, Scheich, & Freeman, 2001). Furthermore, silent problem solving strategies may be taking place during resting state, in the form of insight processes reflected in the spectral properties of EEG lateralized in the right hemisphere. Another significant source of support for the interpretation of resting state as a marker of cognitive performance comes from studies reporting associations between intellectual and cognitive level with certain parameters of brain network configuration at rest, such as small-world or clustering coefficient (Douw et al., 2011; van den Heuvel, Mandl, Kahn, & Hulshoff Pol, 2009), which will be introduced in posterior chapters of this work, or with brain spontaneous connectivity between certain regions (Song et al., 2008).

An extensive line of evidence has demonstrated that brain activity during resting state is organized in such a way that certain regions tend to show

similar trajectories of co-activation. These sets of regions are known as resting state networks (RSNs) and they are known to be consistent across subjects, and also across time for the same subject (Damoiseaux et al., 2006). The first RSNs studies found two sets of brain regions: one of them showing task-related activations, and the other exhibiting task-related deactivations (Fox et al., 2005). These two initial anti-correlated networks led to the discovery of multiple subnetworks from these broad original sets of regions, which relate to different cognitive and sensorimotor functions, like the motor network (Biswal, Zerrin Yetkin, Haughton, & Hyde, 1995), visual and language networks (Cordes et al., 2000) and attentional networks among others (Zhao, Li, Uono, Yoshimura, & Toichi, 2017). The task-negative network most known as default mode network (DMN) has attracted a lot of attention in resting state research. DMN includes areas such as cingulate cortices, inferior parietal and medial prefrontal regions and exhibits higher levels of activation during rest than during task performance. DMN activity is typically related with stimulus-independent internal thoughts (Mason et al., 2007). DMN has also been linked to internal processing, linking already stored personal experiences with planning and external word monitorization (Buckner, Andrews-Hanna, & Schacter, 2008).

Given that resting state structure and dynamics can unveil information about cognitive function in normal healthy states, it is a reasonable assumption to consider that it might inform about brain pathologies. In fact, resting state abnormalities have been largely reported as biomarkers of Alzheimer´s Disease (AD) and have been associated with cognitive progression throughout the course of the illness. For instance, the characteristics of resting state power spectrum (Vecchio et al., 2013) or the intrinsic connectivity between DMN regions (Greicius, Srivastava, Reiss, & Menon, 2004) are known to be affected in AD. Although of capital importance for this thesis, AD is not the only disease in which resting state can be used as a biomarker for diagnosis. Enhanced gamma activity (White & Siegel, 2016) or disruptions in delta and theta bands (Di Lorenzo et al., 2015) have been identified as prototypical signs of schizophrenia. Also in major depression alterations in the resting state profile are used to predict disease severity and progression (Jiang et al., 2016). Following this line of evidence, the present thesis examines resting state patterns in preclinical AD patients to distinguish and characterize possible AD markers in the very early stages of the disease

1.2. Dementia and Alzheimer´s Disease

1.2.1. Dementia: definition and subtypes

Dementia is not a disease itself, but rather a group of symptoms caused by a number of conditions. Dementia is a wide term that can reflect several conditions, although in general, it involves difficulties in cognitive processes such as thinking, remembering and reasoning, which affect patient´s ability for independent daily living. Dementia-like symptoms can be temporarily caused by different medical conditions, such as depression, certain types of medication or schizophrenia among others. However, it is important to rule out these possible confounders of dementia to establish a correct diagnosis. The American Psychiatric Association (APA) has recently proposed replacing the term dementia for major neurocognitive disorder, furthermore mild cognitive impairment (MCI) has been renamed as minor neurocognitive disorder (Regier, Kuhl, & Kupfer, 2013) in an attempt to remove the stigma surrounding dementia. Besides labelling changes, some diagnostic criteria have been modified from DSM-IV-TR (APA, 2002) to DSM-5, as shown in Table 1-1. Most importantly, cognitive impairment as a dementia criterion is no longer necessarily limited to memory deficits as it was in DSM-IV. On the contrary, significant deficits in any of the proposed cognitive domains are now sufficient to fulfill criterion A of DSM-5. From now on we will refer to this condition with the classic term dementia, as it is the one mainly used in current research and clinical scenario (Liu et al., 2017).

It is important to bear in mind that healthy aging is also usually associated with normal changes in cognition, especially in processing speed, attention, cognitive flexibility, visuospatial skills and memory. Thus distinguishing normal declines in cognitive functioning associated with healthy aging from pathological impairment is crucial. Dementia has been defined as an acquired organic pathology able to produce a persistent impairment of superior mental skills leading to a functional deficit in social as well as working environment in people with no awareness deficit (Alberca & López-Pousa, 2006).

Dementia can be classified attending to different characteristics of the disease (Meilán & Gutiérrez, 2017), such as:

- Pathology localization: Regarding the regions affected by the underlying pathology, dementias can be classified as either cortical or subcortical, depending on whether cortical surface of the brain or deeper structures are mainly affected.

DSM-IV criteria for dementia	DSM-5 criteria for major neurocognitive disorder
A1. Memory impairment	**A.** Evidence of significant cognitive decline from a previous level of performance in one or more cognitive domains: - Learning and memory - Language - Executive functions - Complex attention - Perceptual-motor - Social cognition
A2. At least one of the following: - Aphasia - Apraxia - Agnosia - Disturbance in executive functions	**B.** The cognitive deficits interfere with independence in everyday activities. At a minimum, assistance should be required with complex instrumental activities of daily living, such as paying bills or managing medications.
B. The cognitive deficits in A1 and A2 each cause significant impairment in social or occupational functioning and represent a significant decline from a previous level of functioning	**C.** The cognitive deficits do not occur exclusively in the context of a delirium
C. The cognitive deficits do not occur exclusively during the course of delirium	**D.** The cognitive deficits are not better explained by another mental disorder (*e.g.*, major depressive disorder, schizophrenia)

Table 1-1. Criteria for dementia and major neurocognitive disorder in different DSM versions

- Etiology: Depending on the cause of the disease, dementias can be considered: degenerative (if they are caused by a degenerative disease such as AD), infectious, vascular, metabolic, deficitary (*e.g.* caused by a B_{12} vitamin deficit), traumatic or toxic.

- Curability: Dementias can either be classified as reversible or irreversible.

- Onset: Dementias can be classified as acute if the onset is sharp or chronic with a slow and insidious onset.

Evolution: According to the stage of the disease dementias can be termed as mild, moderate or serious.

The most frequent types of dementias as well as their symptoms and characteristics will be briefly introduced in this section (Figure 1-8 presents prevalence of each dementia cause). However, forthcoming sections present a deeper description of AD, as it represents the main focus of the present thesis.

- Alzheimer´s Disease: It is the most common cause of dementia accounting for about 60 to 80% of total dementia cases. AD is a neurodegenerative pathology discovered by Alois Alzheimer in 1907, who described its symptomatology and pathology for the very first time. It is mainly characterized by prominent memory impairment. The codification of new memories is especially compromised in the early stages, although it can also produce confusion, mood disorders and spatio-temporal disorientation. The hallmark AD pathophysiology involves the progressive accumulation of beta-amyloid plaques outside neurons and twisted tangles of protein tau inside neurons. AD pathology advances slowly and insidiously and the first signs of the disease might appear as late as 20 years after the onset of brain pathology (Edmonds et al., 2016; Jack et al., 2009). Consequently, the first cognitive symptoms may go unnoticed until years later in disease progression. AD diagnosis has been traditionally employed only when a patient already exhibited dementia symptoms. However, the recent discovery and popularization of sensitive biomarkers as early signs for an accurate detection of AD has allowed the diagnosis of prodromal stages of the disease (either termed as MCI due to AD or even preclinical AD).

- Frontotemporal dementia: Frontotemporal dementia usually onsets before common senile age, typically ranging between 45 and 65 years age. The mean duration of the disease ranges around 8 years but its progression is also slow and insidious in the beginning. Both gray and white matter are heavily affected, especially in prefrontal and temporal pole regions. There is a pronounced cortical shrinkage and presence of tau protein accumulation. Unlike in AD progression, memory is relatively preserved in the early stages of the disease. On the contrary, personality and behavior are markedly affected as well as language production and comprehension. Typical memory questionnaires for cognitive impairment such as MMSE (Mini–Mental State Examination)have proven to be relatively insensitive

to the early cognitive changes observed in this type of dementias (Gregory, Orrell, Lecturer, Sahakian, & Hodges, 1997), although it tends to drop faster after diagnosis in frontotemporal dementia compared to AD (Chow, Hynan, & Lipton, 2006). Neuropsychological assessment is especially affected in classical executive functioning tasks such as Trail Making Test and oral or design fluency tasks. Frontotemporal dementias account for 10% of total dementia cases approximately, however, it is as prevalent as AD in patents between 45 and 65 years (Ratnavalli, Brayne, Dawson, & Hodges, 2002).

- Vascular dementia: Cognitive vascular diseases represent an amalgam of syndromes with a common feature; all of them present a vascular component in the pathogenesis of the disease. A critical aspect in this type of dementia is that cardiovascular factors represent a somewhat controllable risk variable, thus therapies focused on prevention and life habits modification may play an important role in the progression of this specific type of dementia. Vascular dementia is more frequent between 60 and 70 years old and it is more prevalent in men than women. Furthermore, it presents a familiar pattern in 15 to 30% of the total number of cases (Meilán & Gutiérrez, 2017). Clinical studies report that vascular dementia represents a 10-15% of total dementia cases (de Bruijn et al., 2015). However, it has been recently suggested that this figure could possibly be smaller, as post-mortem studies showed that only 2% of the cases showed vascular disease as the only morphologic cause of dementia, as cited by Perneczky et al. (2016). Nevertheless, before the changes made in DSM-5,the inclusion of memory impairment as a mandatory requirement for dementia diagnosis reduced the number of vascular dementia cases detected, as a considerable amount of patients initially develop executive functioning deficits leading to daily life impairment (Weiner, 2012).

Because of the difficulties to correctly diagnose vascular dementia, structural neuroimaging techniques result pivotal in the clinical process. Most of the cases evolve slowly over several years through the accumulation of small vascular and microvascular lesions mainly over subcortical regions. Furthermore, PET can also help distinguishing this dementia from AD, as vascular dementia patients do not show signs of hypometabolism, contrarily to AD patients.

The cognitive deficits typically follow a tiered progression in multi-infarct dementia. The progression of the disease is commonly slow and it affects not only cognition but also mood regulation, behavior and motor abilities. The first symptoms are typically related with mood and behavioral disorders and urinary problems. The most common cognitive changes in vascular dementia involve attentional deficits, processing speed and executive functioning impairment, although these clinical manifestations

are obviously mediated by the precise location and extent of the vascular lesions.

- Synucleinopathies: Dementia with Lewy bodies and Parkinson´s Disease: This group of pathologies is characterized by the presence of α-synuclein deposits in specific neural and glia populations. The most common dementia due to synucleinopathy is Dementia with Lewy bodies. Patients typically present some of the symptoms characteristic of AD, although in the early stages sleep-related problems, visual hallucinations, visuospatial impairment and some parkinsonian symptoms such as slowness or gait imbalance are more commonly reported. Nevertheless, memory deficits are evident throughout the course of the disease. Lewy bodies are abnormal accumulations of the α-synuclein in neurons, particularly affecting frontal subcortical structures giving raise to deficits in executive functioning and visuospatial deficits. Lewy bodies' pathology can lead to dementia on its own, but this pathology is frequently accompanied by the coexistence of AD pathology and even vascular burden, which often leads to confusion in the diagnostic process.

Parkinson´s disease has not been traditionally associated with cognitive deficits. However, nowadays it is clear that cognitive decline is one of the most weakening aspects of this disease, and up to 15% of the patients older than 70 progress to dementia each year (Galvin, 2006). Dementia due to Parkinson produces cognitive and motor slowness, disexecutive syndrome as well as memory retrieval deficits. Dementia prevalence in patients affected by Parkinson disease is 2 to 6 times greater than in healthy matched controls (Galvin, 2006). α-synuclein deposits in Parkinson disease tend to appear in a deep structure called substantia nigra, which is mainly in charge of brain dopamine production.

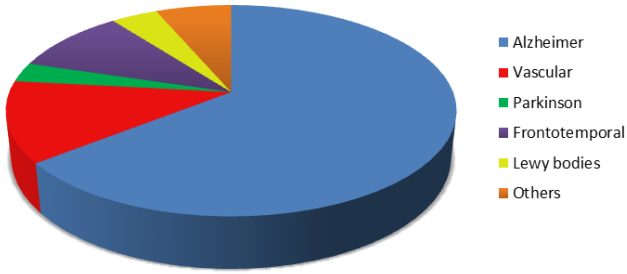

Figure 1-8. Prevalence of the different causes of dementia

- Mixed dementia: Some dementia cases are caused by more than just one disease. The brains of such patients have pathological signs of two or more

typical cases of dementia. The most common mixed cause of dementia is AD and vascular burden, followed by AD in combination with Lewy bodies (Schneider, Arvanitakis, Leurgans, & Bennett, 2009). According to this study, in more than a half of dementia cases, the etiology could be related with more than one disease.

1.2.2. Neuropsychological changes in AD

AD pathology mainly affects cortical structures. Particularly, memory related areas such as medial temporal structures are affected in the early stages of the disease, giving rise to some degree of anterograde episodic memory impairment, consisting in a difficulty to create new memories, rather than retrieving old ones. This impairment in memory consolidation worsens over the course of the disease. In fact, in later stages, patients also suffer from retrograde amnesia: they experience difficulties in retrieving old material. Semantic memory is also impaired and naming task performance is dampened.

Executive functioning is affected also in the early phases of the disease. More concretely, AD pathology produces marked deficits in selective and divided attention, inhibition of external interference and working memory (Kirova, Bays, & Lagalwar, 2015).

Semantic deficits underlie initial language problems in AD. Naming as well as oral fluency performance (especially semantic fluency) might be altered even in the prodromal stages of the disease. Even though reading and writing are relatively preserved in the initial stages, their trajectory throughout the course of the disease tends to decline alongside speech abilities. Oral language becomes poor and aphasic with pronounced difficulties to find the correct words.

AD is also known to produce imitative and gesture apraxia, with notable difficulties in tool use (Baumard et al., 2016) that have been related to the above-mentioned semantic deficits. Furthermore, visuospatial deficits are also typical as AD progresses and can be detected in figure copy or constructive tasks and even produce visual agnosia in later stages.

1.2.3. Neurobiological basis of AD

Alzheimer is a very slow and insidious disease as already mentioned causing progressive cognitive deficits and ultimately dementia. Brain pathology is known to accumulate for decades before cognitive symptoms onset. Several hypotheses have been introduced, which argue for different neurodegenerative mechanisms associated with AD origin and progression. The amyloid cascade hypothesis (Figure 1-9) has been by far the most studied and documented premise trying to explain and predict AD

progression (Jack et al., 2010). According to this theory, the incorrect processing of amyloid precursor protein (APP), which is responsible for the generation of amyloid beta protein (Aβ), combined with a deficit in its clearance would result in the accumulation of Aβ in the synaptic space outside neurons, eventually leading to the formation of protein plaques. From this point, cascading failures in downstream biochemical processes would result in the accumulation of fibrillary tau in neurons, cell death and eventually synaptic dysfunction, leading to cerebral atrophy and cognitive deficits (Pákáski & Kálmán, 2008). A recent data-driven analysis sequencing biomarker alteration onset throughout AD progression support the predictions made by Jack et al. and the amyloid hypothesis (Young et al., 2014).

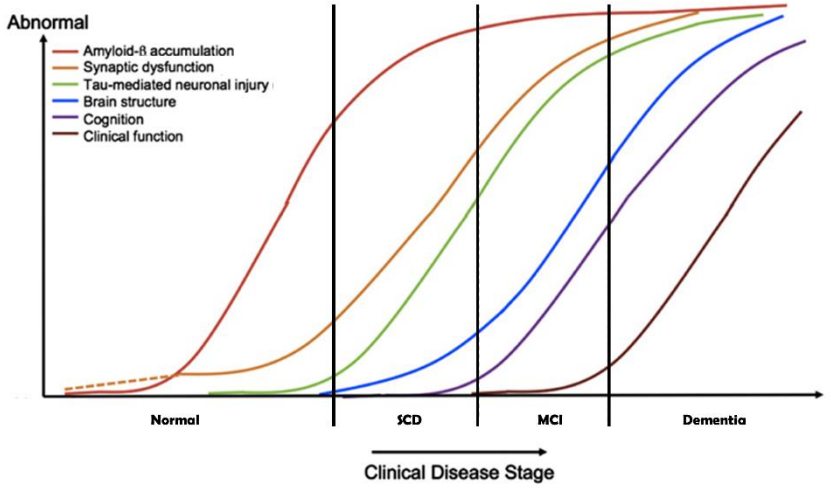

Figure 1-9. Progression of the biomarkers progression according to the Amyloid Cascade Hypothesis. Modified from (Sperling et al., 2011)

Besides amyloid burden, several other factors are known to affect the course of the AD. The other major hallmark of AD pathophysiology is tau protein. The excessive phosphorylation of this protein is responsible for the breakdown of microtubules in the affected neurons, thus provoking axon rupture (Alonso, Zaidi, Grundke-Iqbal, & Iqbal, 1994). Additionally, phosphorylated tau only shows low microtubule-promoting activity but interacts with normal tau, also dampening its normal functioning. This abnormal tau form damages cytoplasmatic functions thus leading to cell death (Mudher & Lovestone, 2002).

Vascular pathology is also known to contribute to AD progression. According to the vascular hypothesis AD would appear as a result of the convergence of two known risk factors for the disease, age and vascular burden. The resulting metabolic demands would result in cellular pathology

involving protein abnormalities, the appearance of plaques and more AD-related pathology (Di Marco et al., 2015). According to this theory, an initial decrease in cerebral perfusion promoted by aging and cardiovascular risk factors would produce reduced metabolic supply (oxygen, glucose, etc.), damaging neuronal populations and the blood-brain barrier, which is responsible for blood flow regulation, creating a vicious circle of further cerebral perfusion alterations. According to the two-hit vascular hypothesis, microvascular damage is the initial alteration leading to blood-brain barrier disruption and resulting in a secondary neuronal damage that would ultimately facilitate the accumulation of Aβ, thus initiating the amyloid cascade.

Inflammation via microglia and astrocytes is also implicated in AD pathogenesis. Aβ deposits can activate microglia, resulting in the release of certain inflammatory substances as well as tumor necrosis factor. The combined effect of these substances and the phagocytic response also induced by Aβ results in neuron damage and cell death contributing to AD progression (Moore & O'Banion, 2002).

AD has been also associated with other pathological processes such as free radical production and a deficient antioxidant capacity leading to cellular damage. This has been termed the oxidative stress hypothesis in AD (Aliev et al., 2006). Furthermore, changes in cholesterol, and insulin have also been proposed as possible mechanisms underlying or at least modulating AD etiology (Carro & Torres-Aleman, 2004; Wellington, 2004).

Neuroimaging techniques can be used to characterize most of these brain alterations and track clinical progression throughout the disease progression. For example, Aβ accumulation is usually studied using PiB-PET (section 1.1.2.). AD patients usually show higher radionuclide uptake over frontal, parietal and lateral temporal regions, and particularly in the posterior cingulate cortex (Kemppainen et al., 2006). However, in a longitudinal study, the rate of change in PiB retention did not correlate with cognitive deterioration (Jack et al., 2009). This study supports the idea that amyloid accumulation is not the direct cause of cognitive impairment in AD. On the contrary, in the same study, the rates of structural impairment (measured as ventricular volume increase) clearly paralleled the changes in cognitive performance in AD patients. Structural atrophy in AD patients is usually found in medial temporal structures such as bilateral hippocampi, amygdala and entorhinal cortex (Matsuda et al., 2012). Furthermore, the above-mentioned hypoperfusion together with its consequent metabolic decrease and eventual neuronal death have been addressed using FDG-PET. These studies typically report a decrease in glucose metabolism over bilateral posterior cingulate cortices and parieto-temporal regions (Kawachi et al., 2006). Tau lesions can also be detected using PET. In fact, tau PET

has been shown to correctly detect tau deposits over hippocampal and frontal regions (Fodero-Tavoletti et al., 2014) as well as in the temporal cortex in AD patients (Lemoine et al., 2015).

Synaptic alterations have a slightly posterior onset within the AD pathological process compared to tau tangles and Aβ plaques. Synaptic disruption in AD is observed as an alteration in the communication pattern between distant brain regions. Functional connectivity is altered in AD patients not only during the performance of cognitive tasks (Grady, Furey, Pietrini, Horwitz, & Rapoport, 2001), but also during the resting state (Allen et al., 2007; Wang et al., 2006). This impairment of the normal temporal coordination between brain structures has led to the categorization of AD as a disconnection syndrome (Bokde, Ewers, & Hampel, 2009). For instance, decreased connectivity from the posterior cingulate cortex (PCC) to several cortical regions has been consistently reported (Weiler et al., 2014). These functional connectivity studies highlight the relevance of PCC within the AD pathology, and are in line with the above-mentioned hypometabolism changes. In fact, PCC is a key structure of the DMN, whose functional connectivity (FC) impairment has been consistently associated with AD progression (Jones et al., 2011; Kim et al., 2016). The vulnerability of brain networks to damage in key regions for brain communication, such as the hippocampus, or posterior regions of the DMN, have been shown using multilayer MEG analyses (Yu et al., 2017). The spectral properties of AD brain rhythms are also known to be affected. Particularly, slow frequencies (delta and theta bands) usually show increased power in AD patients compared to controls, while faster rhythms (alpha, beta and gamma) show decreased power (for a review see, Engels et al., 2017).

1.2.4. Risk factors for AD

Demographic factors

Age is beyond doubt the most predictive risk factor for AD. In a longitudinal study with more than 6000 subjects, each additional year over the age of 70 increased the risk of developing AD by 23% (Lindsay et al., 2002). The incidence rate for dementia increases exponentially with advancing age, and the biggest increase occurs during the 7^{th} and 8^{th} decades of life. Gender seems to be associated with dementia risk; however, this fact is less consistent in literature. Some studies found a 2-fold increase in dementia risk in women compared to men, (Meilán & Gutiérrez, 2017), although other studies did not find any gender difference (Lindsay et al., 2002). Increased risk in women could be mediated by interactions in the expression of other risk factors such as APOE genotype (Altmann, Tian, Henderson, & Greicius, 2014).

Genetic factors

Early-onset AD represents a minor proportion of the total Alzheimer cases (around 1-5%). However, this early form, characterized by a faster decline and worse progression, is clearly associated with genetic factors and presents a Mendelian pattern of inheritance. Three different genes related with APP encoding have been largely associated with the high heritability of early dementia form (APP, PSEN1 and PSEN2). They are mostly autosomal dominant and are certainly associated with Aβ aggregation.

When considering sporadic late-onset AD dementia, familiar history still plays a significant, yet not critical, role. First-degree relatives of AD patients are known to have a 2-fold increased accumulated risk during life of developing AD. The best characterized gene contributing to AD heritability is APOE. APOE can present three different isoforms: APOEε2 APOEε3 and APOEε4. A meta-analysis revealed that, among the Caucasian population, having at least one ε4 allele increased the risk of AD by 2-3 times, while having both ε4 alleles increased this risk by a 14 factor (Farrer, 1997). While APOEε4 is the strongest genetic predictor of late-onset AD, there are several other contributing genes identified in genome-wide association studies that exert a smaller although significant modulation of AD risk: CLU, PICALM, CR1 or BIN1 among others.

Cardiovascular factors

Cerebrovascular disease such as micro or macroinfarcts, vasculopathies and white matter hyperintensities increases the risk not only for cerebrovascular dementia but also for AD. This vascular pathology could either directly affect key regions for cognitive processing, thus impairing cognitive functioning, or promote cytotoxicity increasing Aβ accumulation and inflammatory responses. Other vascular variables such as high blood pressure during midlife (40-60 years old) have proven to be consistently associated with increased AD risk (Whitmer, Sidney, Selby, Johnston, & Yaffe, 2005). Hypertension may affect the blood brain barrier by increasing protein deposition in brain tissue. Some studies pointed that hypertension could play an inverse role if it occurs only in advanced age, possibly because it counters the hypoperfusion known to increase AD risk because of metabolic deficits (Reitz & Mayeux, 2014). Cholesterol levels also seem to modulate AD risk and some studies reported increased AD prevalence in subjects with high cholesterol levels during midlife (Kivipelto et al., 2001). The possible mechanisms behind this association could either be linked to cerebrovascular damage produced by atherosclerosis or increased Aβ accumulation mediated by cholesterol levels (Imtiaz, Tolppanen, Kivipelto, & Soininen, 2014). A number of studies have reported a strong implication of cardiovascular risk factors in about a third of total dementia cases (Barnes & Yaffe, 2011; de Bruijn et al., 2015), which indicates that a great

proportion of dementia cases could be avoided by successfully controlling cardiovascular pathology.

Cranial traumatism

Although some studies have failed to find a direct relationship between head trauma and risk for AD (Lindsay et al., 2002), there is a considerable body of literature reporting a positive association (Fleminger, Oliver, Lovestone, Rabe-Hesketh, & Giora, 2003; Mayeux et al., 1995; Rasmusson, Brandt, Martin, & Folstein, 1995). Apparently, head trauma could increase on its own the deposition of Aβ and tau proteins in brain tissue, thus fostering AD pathogenesis (Franz et al., 2003).

Lifestyle factors

Education is one of the most commonly associated lifetime variables with the risk of developing AD. Fewer years of formal education have been associated with increased risk of developing dementia (Ngandu et al., 2007). Even in susceptible population, such as APOEε4 carriers, higher educational levels have been related to a lower dementia incidence (R Buckley et al., 2013). This protective effect of education and similar variables has been called cognitive reserve (Tucker & Stern, 2011). According to some studies, cognitive reserve does not influence the levels of neuropathology in the individuals, but rather modulates their ability to cope with that neurodegeneration, delaying the onset of clinical symptoms for a given level of pathology (Brayne et al., 2010).

Physical activity has been also traditionally associated with a reduced risk of cognitive impairment and dementia. More concretely, physical exercise during midlife is thought to improve cardiovascular conditions, which in turn reduced dementia risk during aging, as shown in a longitudinal study (Chang et al., 2010). Interestingly, some studies have shown that not all types of physical activity lead to improved health during aging. According to Rovio et al., (2005), physical activity linked to leisure activities during midlife, and not physical exercise associated with work (Rovio et al., 2007) led to reduced risk of cognitive impairment and dementia. Potential mechanisms behind this protective factor could be attributed to increased cerebral blood flow, improved oxygen and glucose utilization, and increase of growth factor release (Reitz & Mayeux, 2014).

Other modifiable habits such as dietary pattern have been associated with dementia risk. Consumption of saturated fats leads to increased dementia rates according to (Barberger-Gateau et al., 2007). Contrarily, adherence to balanced diets such as the Mediterranean diet reduces those rates (Scarmeas, Stern, Tang, Mayeux, & Luchsinger, 2006). Habits such as smoking or excessive alcohol intake can also increase dementia incidence.

However, alcohol have shown a dose-dependent effect, as moderate consumption seems to reduce AD risk (Panza et al., 2012).

1.2.5. Socioeconomic impact of AD

All neurodegenerative diseases, and in particular AD considering its high prevalence, result in progressive cognitive and psychiatric impairment and eventually imply the necessity of medical assistance and caregiving. This comes at an enormous socio-economic cost (Figure 1-10). It is noteworthy that the age-specific risk for developing AD and other dementias has apparently declined over the last 25 years (Matthews et al., 2013).

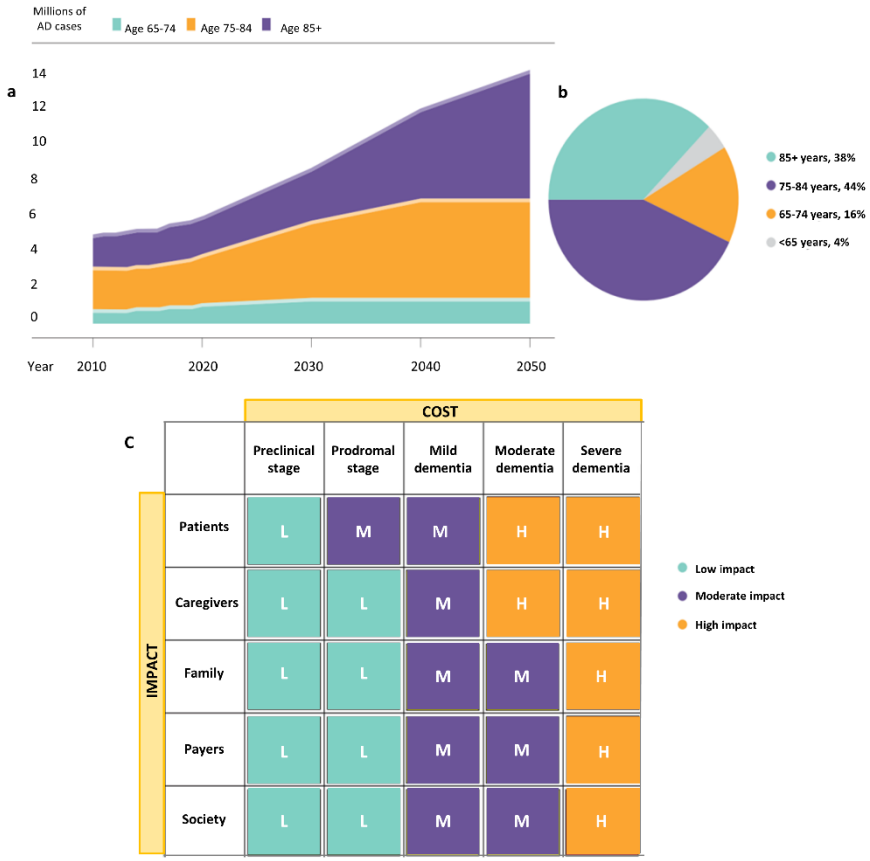

Figure 1-10. Sociodemographic and economic data about Alzheimer´s Disease. (a) Expected population numbers for different age ranges in the US from 2010 to 2050. (b) Age distribution in people with AD in the US in 2017. (c) Economic cost in the AD-continuum. (a) and (b) are reproduced from Alzheimer Association, (2017). (c) is adapted from Jönsson et al., (2017)

This delay in the onset of disease could be related to the increase in education level as well as the improvement in the prevention of cardiovascular pathologies. However, the total estimated cost of AD around the world in 2015 was US$ 818 billion, and it is expected to double in the next decade (Jönsson, Lin, & Khachaturian, 2017). These apparently discrepant results are in fact reconcilable attending to the evolution of modern societies, which age increasingly (Figure 1-10). With the improvement of social and medical care, the number of people above 80 years old, which have a high risk of AD, is expected to increase dramatically. Furthermore, the worldwide increase in prevalence of other AD risk factors such as obesity and diabetes mellitus will likely trigger an increase in dementia incidence in future decades (Hickman, Faustin, & Wisniewski, 2016). Consequently, even though the AD onset is being delayed in an individual's lifetime, the amount of elderly people and the worsening of certain risk factors in our society is expected to lead to an actual increase in the prevalence of AD. AD is a devastating illness, causing an enormous amount of deaths and quality life reduction. Considering all the above-mentioned, it is pivotal to detect and treat AD as soon as possible. The decrease in the age-specific risk of AD in recent decades suggests that interventions modifying life-habits and risk factors may produce a significant change in a subject´s risk to develop AD. Consequently, being able to identify at-risk populations, in which preventive treatment could be specifically recommended, may drastically improve cost-effectiveness of these treatments. In this line, the present work, and others, seek to identify reliable and sensitive early markers not only in AD, but also in predementia stages that may possibly contribute to early detection and eventually intervention (Wimo et al., 2014).

1.2.6. Mild cognitive Impairment

Diagnosis and definition

AD has long been treated as a clearly time-bounded clinical entity. In this conception, Alzheimer´s Disease was just diagnosed once a subject experienced major cognitive deficits, fitting in what we would nowadays catalogue as dementia due to AD or major neurocognitive disorder due to AD. However, according to more recent guidelines, AD extends through a continuum in which clinical symptoms vary from mild impairment in specific cognitive functions to severe cognitive deficits hindering daily living in the later stages of the disease. Prodromal stages represent the focus of recent research literature, in an effort to detect the disease as soon as possible, before irreparable damages take place. In this context the concept of MCI emerged, which rose to fill the gap in the previous simplistic conception of AD as a dichotomic choice between present when dementia appeared or absent while there were no dementia symptoms. MCI was conceived to represent an intermediate state between healthy cognitive state

and dementia, which was previously undiagnosed (Petersen et al., 2014). This new MCI label helped clinicians to perform secondary preventive interventions.

In the following studies the criteria employed to select MCI patients were based on those published by Petersen (2004). Although posterior versions have refined these criteria, the core remains intact. Overall, MCI patients exhibit deficits in one or more cognitive functions, but their daily functioning is not impaired. Table 2 shows a summary of the diagnostic criteria for MCI.

MCI intends to identify an intermediate state between healthy aging and dementia, not only AD. Furthermore, there is little doubt that MCI patients represent an at-risk population for dementia, as they progress at a 10-15% rate per year to any dementia (Bruscoli & Lovestone, 2004). However, not all MCI patients progress to dementia, in fact, some of them revert to normal cognition (Petersen et al., 2014). Even though MCI patients can progress to any dementia, the presence of memory problems increases the likelihood of developing AD.

MCI diagnostic criteria
Self- or informant-reported cognitive complaint
Objective cognitive impairment in at least one cognitive domain
Preserved independence in functional abilities and daily living
Should not fulfill criteria for dementia

Table 1-2. Criteria proposed by Petersen (2004) to classify subjects as MCI

Pathological findings in MCI

Given the broad definition of MCI as an entrance door to any dementia type, a concrete pathological pattern to all MCI patients should not be anticipated, as there is no common physiological pathology underlying all dementias. However, there is a more homogeneous label: amnestic-MCI (according to the Petersen (2004) classification), or the recently proposed MCI due to AD (the National Institute on Aging and the Alzheimer´s Association (NIA-AA)). This category seeks to specify a concrete MCI pattern specifically prone to develop AD. These MCI patients exhibit similar but less intense signs of AD pathology. Using PET, Edmonds et al. (2016) demonstrated that MCI patients have high levels of amyloid deposition and show an AD-like spatial distribution. Furthermore, MCI

patients typically present patterns of cortical atrophy similar to those exhibited by AD patients (Ma et al., 2016). Although not very specific, the presence of MCI is a good indicator of progression to AD when combined with other indicators such as glucose metabolism reduction for instance (Choo et al., 2013). Synaptic alterations are also thought to exist already in the MCI stage. Several studies have reported altered spectral properties in MCIs compared to healthy age-matched controls. These patterns of alterations are similar to those described in AD: slow frequency power increases (delta and theta) and high frequency power decreases (alpha, beta and gamma) (López, Cuesta, et al., 2014). Additionally, FC alterations have also been described in MCI. In a recent study Garcés et al. (2014) reported that the posterior DMN of MCI patients showed decreased functional and structural connectivity. Importantly, FC alterations at this early MCI stage have proven useful in predicting future conversion to AD in a 2-years longitudinal study (López, Bruña, et al., 2014).

The above mentioned studies highlight that MCI patients are more prone than healthy controls to show AD-like pathophysiological profiles in a variety of measurements, such as amyloid accumulation, gray matter volume loss, metabolism reduction, power spectrum density alterations, FC reductions and white matter abnormalities. All these alterations could account for the increased risk of conversion to AD detected in this population.

1.2.7. Subjective Cognitive Decline

Diagnosis and definition

The concept of MCI has mainly evolved during the last two decades. However, the inclusion of an even earlier stage in the disease progression has occurred only very recently by a recommendation of the NIA-AA (Jack et al., 2011). In this work, authors suggested considering an asymptomatic at-risk stage of AD characterized by the presence of AD biomarkers and the absence of clinical manifestations. Recent literature has postulated that complaints about self-perceived cognitive and daily functioning could be a good indicator of subsequent cognitive decline (Tomaszewski Farias et al., 2017). This stage has received numerous labels in the literature: subjective memory complaints, subjective memory impairment or subjective memory concerns among others. The SCD-I initiative recently proposed to refer to this stage as subjective cognitive decline (SCD), which is defined as a state characterized by the presence of a marked subjective feeling of cognitive worsening (in any cognitive domain, not restricted to memory) and the absence of an objective impairment in neuropsychological assessment (Jessen et al., 2014). The conceptualization of a pre-MCI phase should help finding target populations for pharmacological or behavioral interventions at the earliest possible stage of the disease. It is important to highlight that

the inclusion of SCD as an at-risk state of AD or other dementias is not free of controversy, as multiple studies have established the comorbidity of SCD and personal or psychological traits such as anxiety or depression rather than its relation to AD (Lee, Kang, & Cho, 2017; Tandetnik, Hergueta, Bonnet, Dubois, & Bungener, 2017). Therefore, research in the field of SCD as a presumably preclinical stage of AD is explicitly compelled to control for these psychological traits (Jessen et al., 2014).

In this line multiple studies have shown a clear association between SCD and conversion to dementia, with a 2-fold to 4-fold increase in conversion rates in SCD (Wolfsgruber et al., 2016), and even higher rates in amyloid positive SCD subjects (Rachel Buckley et al., 2016). Some studies put up that external information about cognitive decline might be more accurate and predictive of future progression (Slavin et al., 2015) although other work (Caselli et al., 2014) found that those subjects progressing to MCI or dementia reported a subjective feeling of cognitive worsening on average 30 months earlier than external informants. These findings suggest that external information, although more accurate than subjective reports, could only be sensitive to detect changes later in the disease progression, when symptoms start becoming evident. It is nevertheless important to remark, that the presence of SCD is not a necessary condition for the diagnosis of preclinical AD, nor all preclinical AD should present with SCD (Figure 1-11 shows the hypothetical preclinical-AD continuum).

SCD plus criteria

Core criteria

Subjective decline in memory, rather than other domains

Onset of SCD within last 5 years

Age at onset > 60 years

Concerns (worries) associated with SCD

Feeling of worse performance than others of same age

If available

Confirmation of cognitive decline by an informant

Presence of APOEε4

Presence of AD biomarkers

Table 1-3. Criteria proposed by Jessen et al. (2014) that increase the likelihood preclinical AD pathology in SCD subjects.

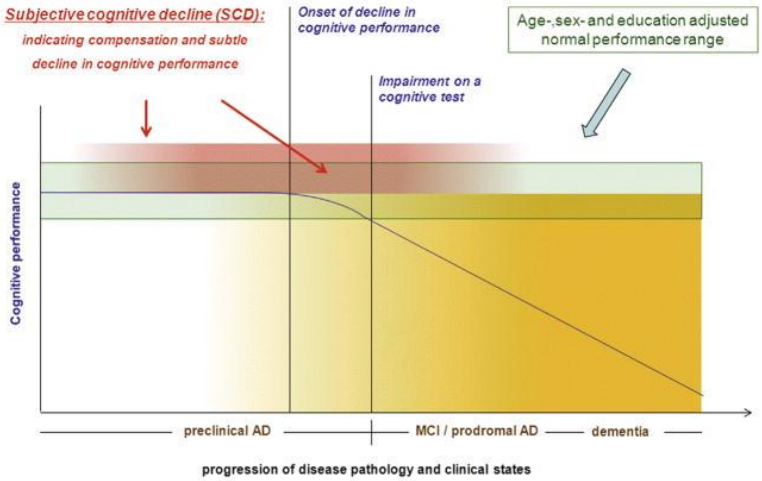

Figure 1-11. Cognitive Impairment progression throughout the AD-continuum (Jessen et al., 2014)

Pathological findings in SCD

Although the concept of SCD has been a subject of some debate because of inconsistent results (Rowe et al., 2010; Tepest et al., 2008), an ever-increasing number of studies coincide in highlighting an augmented proportion of AD pathology in SCD. Although tau levels in plasma did not differ between SCD and healthy control elders (Müller et al., 2017), SCD elders were shown to present an increased probability of having abnormal CSF biomarker levels (Wolfsgruber et al., 2017). Furthermore, SCD cohorts were found to have a higher proportion of Aβ positive and APOEε4 cases than no-SCD cohorts (Fladby et al., 2017). Interestingly, Aβ deposition and cortical atrophy were found to be associated in SCD elders but unrelated in MCI and AD stages (Chételat et al., 2010). This suggests that cytotoxicity and atrophy are only related in the early stages of the AD pathology, and emphasizes the importance of early anti-amyloid interventions. Additionally, SCD elders showed an AD-like pattern of cortical atrophy, including gray matter loss among medial temporal structures such as entorhinal cortices and hippocampus (Peter et al., 2014; Striepens et al., 2010), and signs of hypometabolism over precuneus and temporal areas (Scheef et al., 2012).

However, considering the novelty of the SCD field and the relatively high number of studies probing the presence of AD-like pathology in this group there was a remarkable gap in the literature regarding electrophysiological

alterations in this population. One of the few electrophysiological studies existing prior to the experiments of this thesis examined the synchronization of the functional networks in SCD participants while executing a memory task and reported a slight decrease in their FC strength (Bajo et al., 2012). Lastly, the only study evaluating oscillatory activity in the resting state of SCD elders reported a significant increase of alpha power (Alexander et al., 2007). However this study suffered from serious methodological drawbacks dampening its reliability, as detailed in experiment 1.

1.3. Introduction to thesis methods

1.3.1. MEG components

As introduced in section 1.1.2., magnetoencephalography is a functional technique able to capture outside the scalp the tiny magnetic fields produced by postsynaptic potentials in the cortex. The intensity of these magnetic fields ranges approximately from 50 fT to 500 fT (Hämäläinen, Hari, Ilmoniemi, Knuutila, & Lounasmaa, 1993). Accordingly, the accurate measurement of such small fields requires a specialized technology including an MEG system and a magnetically shielded room. In the next lines we will describe MEG basic functioning. A Vectorview MEG system (Elekta AB, Stockholm, Sweden) was used for the realization of this thesis and some of the particulars will be focused in this concrete MEG system.

Magnetically shielded room

One of the major difficulties of recording brain magnetic activity is its tiny intensity. The first step that should be taken when recording MEG is avoiding or reducing the intensity of external magnetic fields, which represent noise for the MEG recording. Consequently, MEG laboratories try to reduce ambient magnetic noise by settling down in magnetically quiet areas, for instance far away from heavy machinery. However, some sources of magnetic noise are unavoidable. Some representative examples could be the earth magnetic field, which is an intense field with a power law spectral distribution, or the power line artifact produced by electrical devices which is reflected in the MEG power spectra as a prominent and thin peak at 50 or 60 Hz (and harmonics), depending on the country. Remarkably, the intensity of these artifacts is several orders of magnitude bigger than brain´s activity. MEG systems are thus placed inside a magnetically shielded room to reduce this noise and detect brain activity (Figure 1-12). The walls of the room are made of layers of different materials such as aluminum and a nickel alloy called μ-metal. These materials offer a high magnetic permeability, much higher than the interior of the shielded room thus facilitating the conduction of these external fields through the room walls and reducing noise intensity inside the room. Different types of rooms exist, with different designs and resulting shielding power. Although the magnetic noise cannot be completely eliminated, magnetically shielded rooms can attenuate noise between 90 and 130 dB (Gratta, Pizzella, Tecchio, & Romani, 2001). Furthermore, other sources of magnetic are located inside the magnetically shielded room: for instance in the participant or the electronics of the sensors.

Therefore, additional mechanisms to remove unavoidable noise are necessary. These procedures are included in the preprocessing pipeline and will be explained in more detail in section 1.3.3.

MEG sensors

MEG sensors are based on a technology called superconducting quantum interference devices (SQUIDs) that convert magnetic fluxes into voltages. These SQUIDs are formed by a loop of a superconducting element divided in two parts coupled by Josephson junctions,. When a constant current flows through the SQUID, a voltage is generated across the SQUID which oscillates with a characteristic period depending on the magnetic flux entering the SQUID loop, thus allowing the detection of tiny magnetic fields (Kleiner, Koelle, Ludwig, & Clarke, 2004). However, SQUIDs are not employed to directly measure magnetic fields. Magnetic flux is first received by an intermediary sensor with larger area, thus increasing the signal to noise ratio.

There are two types of sensors: the simplest of them is called magnetometer: it consists in a single coil, and measures the component of the magnetic field perpendicular to the coil's surface. Magnetometers are sensitive to magnetic fields generated by both distant and close sources. The second type of sensor is called gradiometer and is formed by combining two coils. In particular, in planar gradiometer the two coils are placed in the same plane, thus measuring the spatial derivative of the incoming magnetic flux in a direction perpendicular to the measured magnetic field. Gradiometers are especially sensitive to close sources. The MEG system employed in the experiments presented in this thesis has 306 channels: 204 planar gradiometers and 102 magnetometers (Figure 1-12). These sensors are grouped into 102 sensor triplets, each containing one magnetometer, and two planar gradiometers (measuring spatial derivatives in the plane tangent to the MEG helmet).

The superconductive property of MEG sensors depends on their temperature. MEG sensors should be placed inside a Dewar filled with liquid helium at a very low temperature (-269 ºC). This Dewar has to be refilled approximately once a week to compensate for the helium dissipation. This makes the maintenance of an MEG system very costly.

1.3.2. MEG recording description

An appointment was individually scheduled for each of the participants to visit the MEG laboratory, located in the Laboratory of Cognitive and Computational Neuroscience in the Center for Biomedical Technology (Madrid). After the participants arrived to the center and signed an informed consent, they filled a questionnaire on their memory concerns. Afterwards each subject was instructed to remove all magnetic objects they wore in order to start the MEG set-up.

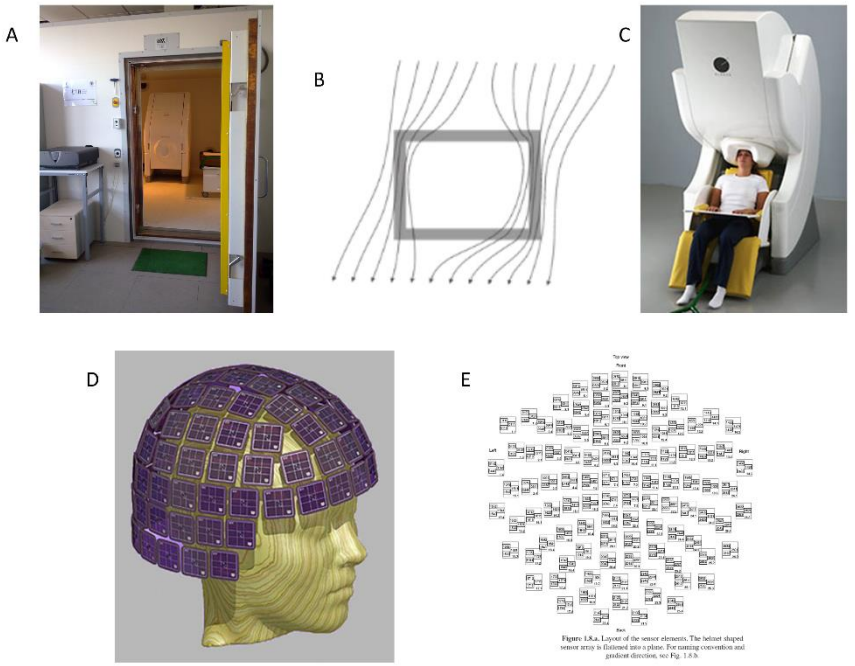

Figure 1-12. Graphical representation of the different MEG components. (A) CTB´s magnetically shielded room. (B) Schematic representation of magnetic fields conductivity in a magnetically shielded room. (C) Electa Vectorview MEG system (D) Spatial 3-D distribution of MEG sensors in our system. (E) Representation in a 2-D plane of MEG sensor distribution

In each recording, three electrodes were attached to control for eye movements. The electrooculogram (EOG) electrodes were placed above and below left subject´s eye and the third electrode, the ground, was placed in the ear. Finally to keep track of head movements during the recording, 4 head position indicators (HPI) coils were attached to participant´s skin; two over the left and right mastoids and two bilaterally over the forehead. These coils produce a magnetic field at 175 Hz so that the head position within the helmet can be continuously determined, and eventually head movements can be corrected. The positions of these HPI coils along with the head shape with respect to the subject's head (defined by three fiducials in left and right pre-auricular and nasion) were digitalized in 3D using a Fastrak Polhemus. The head shape included at least 250 points and it was used to realign MEG recordings to the subject´s MRI scanner in order to achieve a precise source reconstruction (explained in more detail in section 1.3.4).

After the MEG set up was finished, the participant entered the MEG system and performed a memory task (with or without interfering stimuli), a four minutes resting state with eyes open, a 4 minutes resting state with eyes closed, a second memory task (depending on the first memory performed) and finally a passive visualization of faces. The order of these tasks remained the same for all the participants except for counterbalancing of the memory tasks (with and without interference). The three experiments included in this thesis focused on the eyes closed resting state recording. All the recordings were carried out using a 1000 Hz sampling rate, (i.e. acquiring 1000 measurements per second of the magnetic field at each sensor). Additionally, an online band-pass filter between 0.1 Hz and 330 Hz was employed during the recording to avoid aliasing.

1.3.3. Preprocessing pipeline

As already explained in previous sections, the passive shielding against noise is not enough to get rid of all the artifacts affecting MEG recordings. In fact, some of the strongest artifacts are produced by the subject himself in the form of eye movements, head or muscular movements in general, heart beating, etc. As a consequence, it is crucial to process the data in order to remove, or correct those segments containing artifacts which masks brain activity. This procedure is generally called preprocessing and involves a set of steps that can vary between studies. The preprocessing pipeline undertaken in the three studies of this thesis is summarized in this section.

Spatiotemporal signal space separation method (tSSS)

The first step in the preprocessing pipeline is to apply the spatiotemporal extension of the SSS, the so-called tSSS, which was first introduced by Taulu & Simola (2006). The algorithm is included the Maxfilter v2.2. software, native in Elekta systems, and is commonly applied in Elekta MEG recordings (Figure 1-13). SSS assumes the existence of three different volumes: a volume inside subject´s head containing brain currents, an outside volume containing all the currents producing external noise and a current-free intermediate volume containing the sensors. Sensor space signals are decomposed into series expansions of spherical harmonics. The algorithm then projects the components which were found to originate in the inner volume back to the sensor space. The spatiotemporal extension of this filter refines this version by adding the time dimension. Specifically, the algorithm looks for strong correlations between inside and outside components to remove those contributions as the inside components and the externals are expected to be unrelated and independent. It is important to highlight that SSS builds a single set of component using all 306 sensors (magnetometers and gradiometers together). Therefore, after SSS, the information contained in magnetometers and gradiometers is redundant. In

this thesis, all analyses after SSS were performed with the 102 magnetometer dataset. Artifact detection

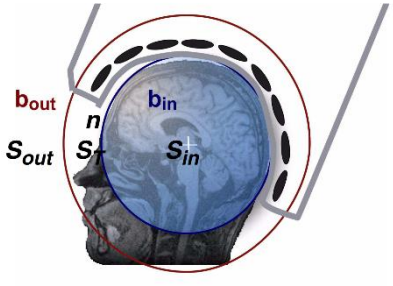

Figure 1-13. Schematic representation of tSSS filter. Inner sphere, S_{in} (fitted to head center) contains the currents that are preserved after filtering. S_{out} is placed outside sensor space and contains the components removed by tSSS. sT is the space near the sensor space and does not contain electrical currents.

After denoising the raw MEG data using tSSS, the data were inspected to detect those segments containing artifacts. In particular, three categories of artifacts were identified (Figure 1-14).

1 - Muscular artifacts: Muscular artifacts are magnetic fields originated by muscle contractions. These artifacts typically show high amplitudes at higher frequencies (>30Hz). Although they are not restricted to a concrete sensor region they are more commonly found over temporal and frontal sensors, due to forehead muscle contractions and jawbone movements among others.

2 – Eye movements and blinks: These artifacts are also associated with muscle movements, although they are specifically associated with eye movements and blinks. Hence, they are typically found over frontal sensors. Eye blinks can be detected in MEG raw data as sudden and discrete changes in amplitude. It is easy ensuring the identification of this type of artifacts due to the EOG channel. Their power spectrum is characterized by a power law distribution, with decreasing amplitude as frequency increases.

3 – Sensor jumps: Jump artifacts are due to failures in sensor electronics and are evidenced as abrupt changes in amplitude. They can be separated from blink artifacts because the latter show a softer shape with a progressive increase and decrease in power rather than a rectangular-like shape.

This artifact detection can either be done using automatized software or visually. In the three experiments included in this volume we employed an automatic procedure from the Fieldtrip toolbox (Oostenveld, Fries, Maris, & Schoffelen, 2011) to detect signal segments containing artifacts. Afterwards, these automatically detected artifacts were visually inspected to ensure the correct classification given the relatively high number of false positive detections.

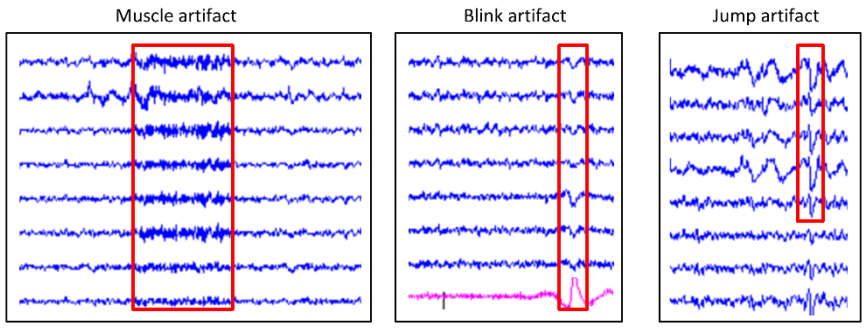

Figure 1-14. Real MEG sensor activity showing typical artifacts. Left image: muscular artifact shown as high frequency and high amplitude oscillations. Middle image: blink artifact registered over frontal sensors and registered in the EOG channel (pink line bottom part). Right image: jump artifact produced by one sensor and spread over more channels because of tSSS signal reconstruction.

After removing all the artefactual segments, clean signal was divided into 4 seconds epochs including 2 more seconds at each side as padding to avoid edge effects in posterior band pass filtering. Only clean trials were used for all subsequent analyses.

Independent component analysis

Even after SSS, and removing notably artefacted segments, MEG signals still contain some noise. Independent component analysis (ICA) is a useful and popular technique to tackle this issue. It separates a multivariate signal into separate independent components. Many physiological and technical sources of noise separate into temporally independent components. This allow for the isolation and identification of the concrete contribution of these artefacts to the MEG signals. In particular, electrocardiogram (EKG), blinks, and ocular movements are usually well captured by ICA. In the three experiments a modification of the typical ICA procedure was employed to detect and remove the EKG component from the signal. This removes their contribution to the MEG signal without affecting the number of clean trials available.

1.3.4. Source space reconstruction

Once MEG raw data have been cleaned of external and physiological noise, the data is ready for the analysis. However, there is still a critical fork in the pipeline. These analyses can be done in the sensor or in the source space. On the one hand, in sensor space analyses, the magnitudes of interest are estimated directly with the data captured by each sensor, and thus the spatial distribution of the possible results is highly compromised due to leakage effect. This effect is produced by the fact that the neuronal activity of a given brain region affects more than a single sensor, blurring the effects. On the other hand, source space analysis disentangles the sources which generate the activity measured at the sensor level. By making use of a variety of reconstruction algorithms and mathematical techniques, the activities of distinct brains locations are reconstructed, thus obtaining the time series of activation of the different regions of interest in the brain. This procedure substantially solves the leakage effect problem mentioned for sensor space analyses, and what is more important, makes MEG a much more transversal functional technique, able to produce results in a significant and comparable framework.

A detailed explanation of the different techniques to reconstruct source activity is out of the scope of this work, but can be consulted elsewhere (Gratta et al., 2001; Maestú, Pereda, & Del-Pozo, 2015; Nolte, 2003; Sarvas, 1987). However, a short explanation on the concrete methods employed follows below.

Forward problem

The calculation of the source space time series requires solving first the so-called forward problem. In this problem the sensor-space magnetic fields resulting from an initially known current distribution in the brain are computed. This mathematical problem implies the calculation of the leadfield matrix containing the relationship between a given activation at each source position (for neuronal currents in each of the three spatial directions *x, y, z)*, and the corresponding magnetic field at each sensor. There are a number of algorithms that allow to calculate a biophysical plausible model of the forward problem. It is worth highlighting that the magnetic field observed outside the brain depends on the particular conductivity profile of the tissues, and that this conductivity value is not constant throughout the brain. Conductivity values for distinct brain tissues have been estimated in previous literature (Haueisen & Knösche, 2012), but the exact head tissue geometry changes across subjects.

In this thesis, the geometry of the skull and scalp was determined for each subject using his/her T1-weighted MRI. Then, the forward model was solved with a boundary element method (BEM), and assuming three

compartments of isotropic and homogenous conductivity: brain, skull and scalp. 3-shell BEM models have proven accurate in estimating realistic leadfield matrices (Stenroos, Hunold, & Haueisen, 2014).

In all the experiments presented here, sources were distributed in a regular 3D grid, with 4560 sources separated by 1 cm from each other into the Montreal Neurological Institute (MNI) space, and transformed to subject space. From these 4560 sources, only 2459 were contained into brain volume, and thus considered for calculations.

Inverse problem

Once the leadfield matrix is obtained (i.e. the MEG signals generated by a concrete current distribution is known), the inverse problem can be tackled. In this step, brain activity (or *source space activity*) is estimated from the MEG sensor measurements.

Contrarily to the forward problem, the inverse problem is ill-posed: it has an infinite number of solutions. To address this, a priori assumptions are needed that restrict the number of possible solutions to a single one. Many assumptions are possible, each giving raise to different solution to the inverse problem, or *source reconstruction* method. Popular source reconstruction methods include dipole fitting (Wood, 1982), minimum norm estimate (Hämäläinen & Ilmoniemi, 1994) or beamforming (Sekihara & Nagarajan, 2008), which we used in the three experiments.

Beamformers are adaptive spatial filters Activity at each source location is calculated as a linear combination of sensor measurements. The coefficients of this linear combinations are given by the spatial filter. They are estimated for each source separately and depend on the leadfield (solution to the forward problem), and on the covariance of the sensor data. A graphical summary of the pipeline followed in this thesis is presented in Figure 1-15.

1.3.5. Main analyses

After source level activity reconstruction, MEG data was set to carry out the planned analyses. In this section the concrete methods and the particularities of the analyses employed in each of the three experiments will be briefly explained.

Power spectrum analysis

Power spectrum analysis is one of the most widely employed approaches to study electrophysiological signals. It is based on the assumption that a complex signal can be decomposed as a sum of sines and cosines with different fundamental frequencies and intensities, in such a way that the

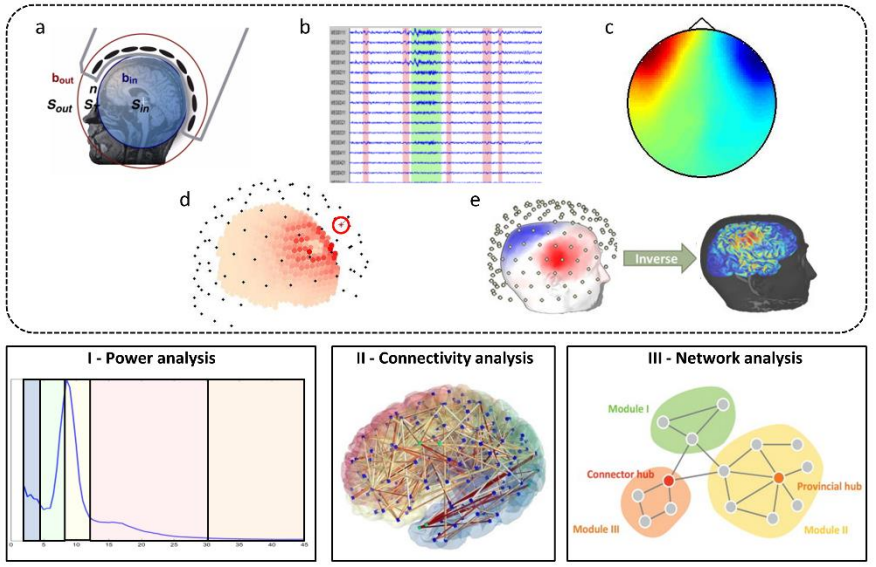

Figure 1-15. Overview of the MEG data processing The upper box shows a schematic representation of the preprocessing pipeline: (a) tSSS of the MEG raw signal, (b) typical artifact detection routine showing a muscular artifact (green) and some blink artifacts (red), (c) topography of the weights of an EOG component extracted using ICA, (d) relative contribution calculated in the leadfield matrix of each source to the activity recorded in a specific sensor (circled in red), (e) inverse problem schematic representation: a time series is calculated for each source in the brain from a sensor space measurement The lower boxes represent the magnitudes of interest used in each of the three main chapters of this thesis, (I) Power spectrum, (II) functional connectivity and (III) graph metrics

contribution of different frequencies to the overall signal can be isolated and measured (Figure 1-16).

Spectral estimation through Fourier transform assumes an infinite length signal, which is not the case with real MEG data. This violation of spectral analysis premises leads to discontinuities in the endpoints of the data, reflected as frequency components in the spectral density results that were not present in the original signal. This effect is called spectral leakage and it results in a smeared spectrum of the original signal (Figure 1-16). This effect can be partially overcome by employing a technique called windowing. By windowing a signal the discontinuities at the boundaries of the finite length signal are minimized. To this end, the original signal is multiplied by a window function, which is a signal that smoothly changes its amplitude from the center to the borders towards zero, thus reducing the contribution of the more extreme points of the original signal to the final spectral result (Figure 1-16).

There are a variety of windowing functions available, each of them having specific characteristics with pros and cons in term of spectral resolution. In

the first experiment of this thesis, spectral properties of MEG brain activity was characterized through multitaper fast fourier transform using discrete prolate spheroidal sequence as tapers. Spectral density was estimated in the range 2-45 Hz at each 0.5 Hz step and 0.5 Hz smoothing.

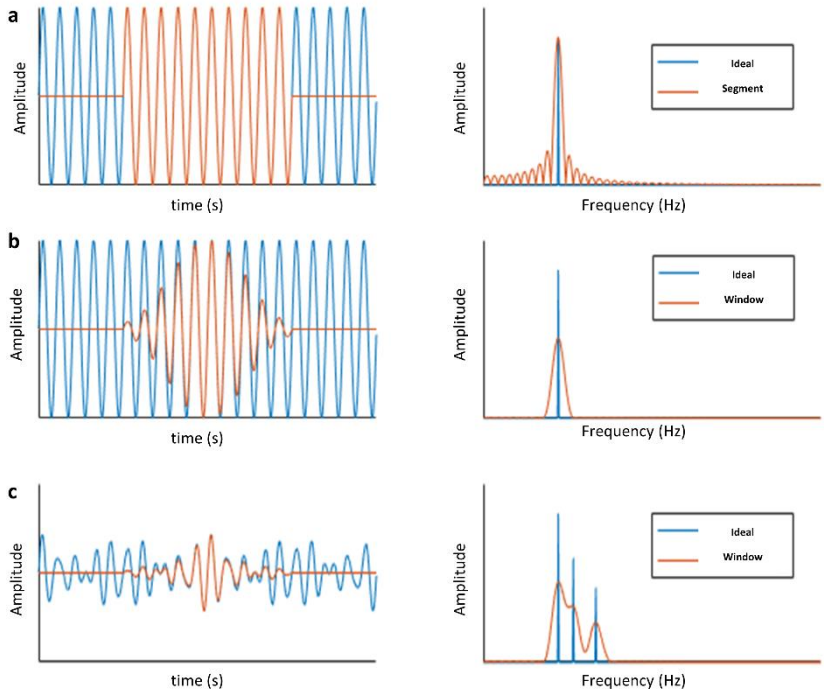

Figure 1-16. Figure shows different oscillations (left) and their respective spectral representation (right). Blue lines represent the ideal scenario of infinite and stationary signals for Fourier transform. Orange lines represent a more realistic scenario in which only a segment of the original signal is analyzed: (a) without a window function obtaining a smeared spectra, (b and c) using a window function obtaining a smoothed peak around each main frequency of the oscillation.

To yield an optimal estimation and comparability of the power spectral density estimation it is often a requisite to normalize its intensity. This can be achieved either by the intensity of a baseline condition for most task paradigms, or by normalising with the overall power intensity over an a-priori known range. When using beamforming source reconstruction, intensity normalization is particularly necessary, as our beamforming source variance estimates are biased and the intensity of deep brain regions is largely overestimated (Barnes, Hillebrand, Fawcett, & Singh, 2004). In the first study the power at each frequency step was divided by the broadband power of the signal, thus yielding a relative power estimate whose integral over the whole frequency range equals 1.

Functional connectivity

The understanding of brain functioning in normal and pathological conditions has always been the main challenge of neuroscience. In this regard, the classical conception of brain functioning brought about by activation studies has demonstrated to be insufficient to explain the complex dynamics and interactions occurring in the brain (Stam & van Straaten, 2012).

These interactions among brain regions represent the cornerstone of the approach of modern neuroscience to understand brain functioning. Connectivity between different regions can be assessed from different angles, depending on whether brain structure or function is being taken into account. According to this, we can distinguish between structural connectivity and FC. Structural connectivity focuses on the white matter fiber tracts connecting distant brain regions, and is usually measured using DTI. The term FC in the context of neuroimaging was introduced to describe the temporal correlation between spatially distant neurophysiological events (Friston, Frith, Liddle, & Frackowiak 1993). This functional coordination is known to underlie every brain process, from simple visual perception (Ekman, Derrfuss, Tittgemeyer, & Fiebach, 2012) to complicated cognitive processes such as memory (Meunier et al., 2014) or emotion (Kinnison, Padmala, Choi, & Pessoa, 2012). However, it is important to bear in mind that extracting causality from simple statistical dependencies and correlation is wrong and should be avoided. The fact that two brain regions exhibit synchronized activity should not be interpreted as one region leading the activity of the second, or even as a direct link between both (Figure 1-17). We can address these questions using effective connectivity measures. Effective connectivity refers explicitly to the influence that one neural system exerts over another, either at a synaptic or population level (Karl J. Friston, 2011). Effective connectivity henceforth determines not only the intensity of the synchronization like FC metrics, but also the direction of such relationships.

In the experiments 2 and 3 of this thesis, FC was evaluated using different metrics. In each of them the functional coupling between brain regions is estimated through different properties of MEG signal, either phase, or amplitude. Both FC approaches are briefly explained below (Figure 1-18).

- Phase synchronization: Oscillatory signals can be transformed into their corresponding analytical signals by means of the Hilbert transform. The phase and the amplitude of the original oscillatory signal can be estimated from the phase and the amplitude of the Hilbert transform. As explained, the in section 1.1.3. the phase represents the relative position of the oscillation within a cycle at a given time, and can be conceived as an angle (measured in rad). Phase locking value (PLV) was used to estimate to

coupling level in experiment 2. It is based on the assumption that measuring the nonuniformity of phase differences across time between two signals is a good estimator of their coupling (Lachaux, Rodriguez, Martinerie, & Varela, 1999). Intuitively, if the phase delay between two timeseries is relatively constant across time, both time series are highly phase locked, and therefore highly functionally coupled. PLV is delimited between 0 (indicative of no coupling between the signals) and 1 (perfect coupling between the signals). In the second experiment we performed source reconstruction for 2459 sources inside the brain, distributed across 64 regions from a reduced version of the anatomical Harvard-Oxford atlas. The FC was calculated between each pair of sources and then averaged across all pairs of sources connecting any two regions of interest, resulting in a 64 x 64 FC matrix for each subject.

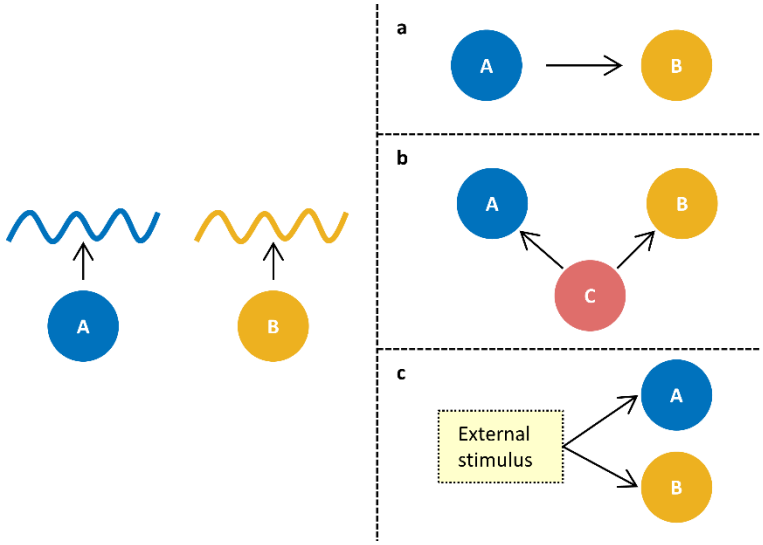

Figure 1-17Schematic representation of two synchronized brain regions (left), and the possible mechanisms underlying their FC (right). (a) A direct link between the two structures drives FC. (b) A third structure directs the activity of the other two, producing synchronization between both regions without a direct link between them.(c) Both regions are engaged in the processing of the same stimulus that drives synchronization without a direct link between them.

- Amplitude synchronization: The envelope of the original signal can be estimated through the modulus of the analytical signal (computed with Hilbert transform). It can be interpreted as the overall intensity at a given time in the frequency band of interest. The FC between two brain regions is here quantified as the Pearson correlation coefficient between both their amplitude envelopes (Brookes et al., 2011). This study also showed that MEG-FC as measured by envelope correlations shows a remarkable

similarity to fMRI-FC maps, and thus seems to be a reliable way to reproduce fMRI findings.

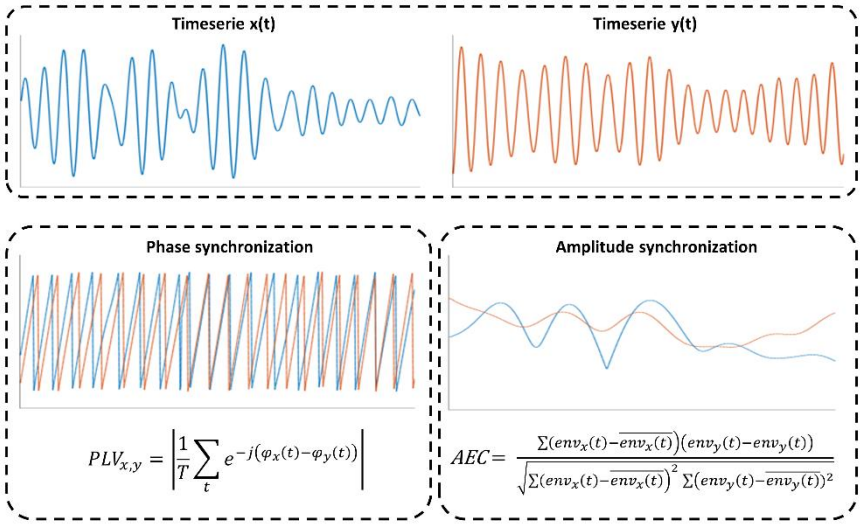

Figure 1-18. The upper part shows the time courses *x(t)* and *y(t)* of two different brain regions. The left bottom part shows time courses of the corresponding phases ($\varphi_x(t)$, $\varphi_y(t)$), and the formula for PLV, the phase synchronization index employed in experiment 2. The right bottom figure shows the time courses of both envelopes ($env\_x(t)$, $env_y(t)$) and formula for amplitude envelope correlation (AEC) used in experiment 3.

MEG FC presents methodological difficulties. One of the main concerns stems from limitations in solving the ill-posed inverse problem: source reconstructions present cross-talk (especially between neighboring sources). This is also termed source leakage, and leads to spurious signal dependencies that might be interpreted as FC. Brookes, Woolrich, & Barnes, (2012) proposed a method to remove zero-lag contribution from the amplitude correlations: orthogonalising source time series before the envelope estimation. This is achieved for each pair of sources separately by regressing out the linear projection of the time series of one source into the other. In experiment 3 we applied this method to construct FC matrices and then calculate graph metrics. The resulting 2459 time courses were grouped into 52 regions of interest (ROI) from a data driven functional atlas (Craddock, James, Holtzheimer, Hu, & Mayberg, 2012). FC between pairs of ROIs was then calculated by selecting a representative source for each ROI, which was defined as the most strongly correlated source on average with all the other sources in the same ROI. This resulted in a set of 52 x 52 FC matrices.

Graph analysis

In the third experiment of this thesis, brain connectivity is studied using a recent framework based in complex systems and mathematical graph theory. This approach characterizes the macroscopic behavior of brain networks from their connectivity patterns. The topological and organizational properties of the network are thought to represent and underlie physiological processes giving rise to cognition and behavior (Bullmore & Sporns, 2009). Furthermore, studying the graph properties of brain networks, is very insightful in the study of both normal brain function and pathological network alterations in AD (Vecchio et al., 2017), schizophrenia (Nelson, Bassett, Camchong, Bullmore, & Lim, 2017) or autism (Keown et al., 2017), among others. Network approaches have the added benefit of providing a general language, allowing the comparison of results from different imaging modalities as their measures characterize network properties than can be more easily translated and compared (Tijms et al., 2013a).

In graph theory, connectivity matrices are reinterpreted in terms of nodes, (i.e. the functional components of the system, in our case anatomical and functional regions of interest) and edges (i.e. the connections between each pair of nodes) (Bassett & Bullmore, 2016). The links between network nodes can be used as weighted measurements of the connection strength, or unweighted, in which case the connectivity matrices are binarized as ones for those edges exceeding a threshold or zero otherwise (Tijms et al., 2013b). In our case, we employed binarized versions of the raw FC matrices in order to avoid dealing with fully connected matrices. This is the approach we followed in experiment 3.

We evaluated 4 graph metrics: smallworldness, clustering, transitivity and modularity to compare network properties of the HC, SCD and MCI groups. Besides, clustering, node degree and module assignment were compared at the node level. These graph metrics are detailed in the methods section of the experiment 3.

2. General objectives

This work includes three main chapters (Experiment I, Experiment II and Experiment III). All of them are focused in the preclinical stages of Alzheimer´s Disease. As already mentioned in the general introduction, AD is a very slow and insidious pathology and all the pharmacological attempts to handle its clinical manifestations have resulted fruitless. The overwhelming social and economic burden of the disease is redirecting research focus towards earlier stages in the pathological cascade. The general objectives of this work, listed below, are framed in this context, with major research efforts to detect early AD biomarkers. In the following lines the specific aspects and questions that this work tackles are introduced. These topics had not been previously targeted in the literature and thus remained completely unexplored. The general objectives of this work are:

- At the time when data collection for this thesis started, very little was known yet about SCD. In fact, it is still a matter of debate whether memory concerns in elderly people represent anything beyond personality traits such as anxiousness or depression. One of the main objectives of this work was therefore to evaluate whether a sample of cognitively intact elders with cognitive concerns (SCD) presented any altered sign in their neurophysiological profile associated with this subjective feeling of cognitive worsening, once personality traits had been ruled out as a possible confounding factor.

- A second objective of this work is to specifically characterize the type of alterations present in SCD population MEG activity. Electrophysiological activity can be approached from very different perspectives. In this thesis an holistic approach was employed to characterize the nature and extent of the brain activity abnormalities in SCD population, if any. In this vein, this work seeks to unravel SCD spectral profile, functional connectivity patterns as well as their network properties.

- From a neuropsychological point of view, SCD elders present intact performance in classical assessment. Only few studies have documented specific neuropsychological deficits in this population such as feature binding deficits in short term visual memory (Koppara et al., 2015). But overall, their memory, executive functioning, language and attention are well preserved, and therefore, they are individually indistinguishable from healthy

elders from a neuropsychological perspective. However, SCD elders as a group, although within the normal range, could perform slightly worse than healthy controls. One of the objectives of this thesis is to relate neurophysiological alterations with these hypothetic slight changes in cognition that current neuropsychological instruments are not sensitive to, and thus, get a hint of the electrophysiological mechanisms underlying early cognitive changes in AD.

- The experiments include not only functional data (MEG) but also structural information (MRI volumetry of AD-related structures). The pathological cascade in AD posits that structural changes in the AD-continuum may appear relatively late. In this context, this work tries to identify the sequence of biomarker appearance throughout the preclinical stages of the disease. More concretely, one of the objectives is to identify if changes at a functional (synaptic) level are present in the SCD group in combination with structural alterations, or before they appear.

- This study seeks to determine whether electrophysiological alterations found in SCD have a connection to AD pathology. Even assuming that SCD present some sort of electrophysiological signature, it should not be necessarily related to AD pathology. As a consequence, one of the main objectives of this work is to identify if these alterations resemble those found in the AD literature. To this aim, the three experiments include a group of patients in MCI stage, which is commonly accepted as a prodromal stage of AD, and has been largely associated with increased AD pathology and progression to dementia.

- Although several neuroimaging studies have reported alterations in the MCI stage, and even some of them using electrophysiological techniques, the latter group of studies is very scarce in comparison with fMRI or PET studies. An additional objective of the present thesis is to replicate some of the previous findings in MCI literature, and even expand previous knowledge by combining source reconstruction and graph metrics with electrophysiological data. More importantly, the following experiments seek to characterize the evolution of brain electromagnetic alterations throughout the early stages of the disease, by describing how these alterations appear from healthy controls to SCD, and how the pathological cascade progresses reaching the MCI phase.

3. Experiment 1

Spectral analysis in SCD and MCI

Publication title:

Alpha band disruption in the AD-continuum starts in the Subjective Cognitive Decline Stage. A MEG study.

Journal:

Scientific Reports

Year:

2016

Authors and affiliations:

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4. Radiology Department, San Carlos University Hospital, Madrid, Spain.

3.1. Summary of objectives

1. This work seeks to elucidate whether healthy elders presenting SCD showed any abnormalities in their spectral profile. More concretely, the analysis was focused in the alpha range as it represents the strongest rhythm in human resting state. The relative power as well as the main frequency of the peak in each source location was compared between groups.

2. The inclusion of an MCI group served the frequently overlooked objective of replicating previous results. Besides, a major objective of this work is to evaluate whether hypothetical changes observed in SCD could represent a pre-dementia stage by comparing their alterations with those found in the MCI group.

3. To evaluate and describe the progression of spectral alterations from a completely healthy state, to MCI. Previous literature remains inconsistent and it is not clear whether changes in the pre-dementia stage follow a linear trajectory with a monotonic increase/decrease in power.

4. To measure the anatomical integrity of a key structure in AD progression as is the hippocampus. This work addressed whether SCD elders presented gray matter loss in this medial temporal structure. Furthermore, MCI volumetric information was also analyzed to replicate previous studies reporting hippocampal atrophy in this group.

5. To disentangle the chronological progression of the different alterations found throughout the AD-continuum. More concretely, the work aimed to answer if power alterations in MEG brain signal or structural alterations appeared first in the pathological cascade of AD.

6. To measure and interpret the possible associations between spectral alterations and cognition in SCD and MCI patients.

3.2. Abstract

The consideration of Subjective Cognitive Decline (SCD) as a preclinical stage of AD remains still a matter of debate. Alpha band alterations represent one of the most significant changes in the electrophysiological profile of AD. In particular, AD patients exhibit reduced alpha relative power and frequency. We used alpha band activity measured with MEG to study whether SCD and MCI elders present these electrophysiological changes characteristic of AD, and to determine the evolution of the observed alterations across AD spectrum. The total sample consisted of 131 participants: 39 elders without SCD, 41 elders with SCD and 51 MCI patients. All of them underwent MEG and MRI scans and neuropsychological assessment.

SCD and MCI patients exhibited a similar reduction in alpha band activity compared with the no SCD group. However, only MCI patients showed a slowing in their alpha peak frequency compared with both SCD and no SCD. These changes in alpha band were related to worse cognition.

Our results suggest that AD-related alterations may start in the SCD stage, with a reduction in alpha relative power. It is later, in the MCI stage, where the slowing of the spectral profile takes place, giving rise to objective deficits in cognitive functioning.

3.3. Introduction

Alzheimer´s Disease (AD) is a growing threat nowadays. Every year around 4.6 million new AD cases are diagnosed all over the world and the number of people that will be affected by 2040 is estimated around 81 million (Ferri et al., 2005). The vast popularity of the amyloid cascade hypothesis has led therapeutic efforts to focus on removing or inhibiting the Aβ plaques formation. However, none of them have shown any improvement so far. This led to a shift in research focus towards the initial stages of AD. The progression of the disease is slow and insidious and the preclinical stage may last several years and start even decades before the first dementia symptoms show up (Jack et al., 2013). Targeting individuals in the asymptomatic at-risk stage of AD with prevention trials may be the most effective approach (Imtiaz, Tolppanen, Kivipelto, & Soininen, 2014) hence there is a growing interest in detecting as early as possible the first signs of AD pathology.

This preclinical stage has been alluded to with different terms in the literature, but it was recently named as Subjective Cognitive Decline (SCD) and a common conceptual framework was settled for research by the SCD-Initiative (Jessen et al., 2014). This stage is characterized by a normal performance on neuropsychological tests besides the subjective perception of worsening in the cognitive functioning. It is also remarkable the presence of AD biomarkers with a very subtle cognitive decline, which can be still compensated thus remaining undetectable by standard neuropsychological testing. However, the idea of SCD representing a very early preclinical stage of the AD continuum has been controversial as memory concerns are highly associated with psychological factors such as personality traits, anxiety or depression (Comijs, Deeg, Dik, Twisk, & Jonker, 2002; Jorm et al., 2004).

However, a growing body of literature seems to support that SCD could be an indicator of early AD pathology. The presence of self-perceived decline has been associated with subsequent cognitive decline and progression to dementia (Gallassi et al., 2010). Additionally, people with SCD present greater rates of incident mild cognitive impairment and dementia compared to normal elders without any perceived cognitive concern (Jessen et al., 2010; van Harten et al., 2013), and its conversion rate per year to mild cognitive impairment (MCI) and dementia is around 6.6% and 2.3% respectively (Mitchell, Beaumont, Ferguson, Yadegarfar, & Stubbs, 2014). Cerebrospinal fluid (CSF) analysis also supports this AD-spectrum hypothesis: SCD subjects present AD-related markers in the CSF (i.e. decreased $A\beta_{42}$ and increased tau) (Visser et al., 2009). Nevertheless, there are still some inconsistent results: while some groups reported reduced hippocampal volume in patients with SCD (Peter et al., 2014; Rabin et al.,

2015), others failed to find differences between SCD and age-matched controls (Jessen et al., 2006). Similar discrepancies have been reported with regard to Aβ depositions in the brain; although some studies showed higher levels of Aβ accumulation (Perrotin, Mormino, Madison, Hayenga, & Jagust, 2012; Rabin et al., 2015) there are some others reporting negative results (Buckley et al., 2013).

Very little is known about the functional organization of the brain in this stage. Participants with SCD exhibited greater brain BOLD responses in fMRI compared to controls in several different paradigms; during memory (Dumas et al., 2013), and attentional tasks (Rodda, Dannhauser, Cutinha, Shergill, & Walker, 2011), and also increased resting state connectivity in the Default Mode Network (DMN) (Hafkemeijer et al., 2013).

EEG and MEG have been proven useful in characterizing different stages of the AD continuum (Babiloni et al., 2006; López et al., 2014). The most characteristic finding regarding the spectral profile of AD is the slowing of the brain rhythms (Neto, Allen, Aurlien, Nordby, & Eichele, 2015). A slowing of the alpha peak has been reported in AD (Samson-Dollfus, Delapierre, Do Marcolino, & Blondeau, 1997) and MCI (Garcés et al., 2013). Furthermore, mean alpha frequency in MEG power spectra is reduced in MCI patients indicating that the beginning of this slowing may take place in the pre-dementia stage (Fernández et al., 2006). MCI patients, both amnestic single and multi-domain show a similar pattern, with an increase in delta and theta power and decremented alpha and beta power (Babiloni et al., 2009; Cuesta et al., 2015; López et al., 2014). Alpha rhythm is of crucial importance. It is the predominant rhythm in the resting brain dynamics and it can be measured over wide regions in the human brain. Besides the power of the band, its main frequency can be easily identified given its prominent nature in the human power spectrum. These two values can draw together an overview of the power spectrum of each group, as alpha peak is the most prominent peak in human power spectrum, thus giving an insight about the time-course of the 'shift to the left' effect described in the AD electrophysiological profile.

Considering all these findings, the use of MEG to study neural dynamics in SCD could provide some insight into the matter. If SCD is actually one of the first stages of dementia, alpha activity could present alterations in line of those reported for MCI and AD (i.e. alpha power decrease or/and alpha slowing). As above mentioned, those neuroimaging studies reporting brain alterations in SCD have mostly reported AD-like pathology or changes in a similar direction but to a lesser extent to those exhibited by AD patients. However, to the best of our knowledge the only study to date reporting resting state power spectral analysis in SCD reported an increase in alpha power both in eyes open and eyes closed condition with respect to controls

without cognitive concerns (Alexander et al., 2006). The authors suggested a nonlinear evolution from healthy controls to AD. However, this is not consistent with different studies reporting AD-like changes in SCD: progressive reduced brain metabolism (Perrotin et al., 2012), progressive increased amyloid burden (Rabin et al., 2015) or brain atrophy (Saykin et al., 2006).

The present study aims to elucidate two main points; 1) whether SCD stage can be considered a pre-dementia stage in which alpha band patterns are disrupted and 2) given the first, we seek to determine the direction of the observed changes (i.e. whether changes follow a linear continuous with MCI-AD or a non-linear pattern). For this purpose we use MEG data from healthy elders without any cognitive concern (no SCD), healthy elders with Subjective Cognitive Decline (SCD) and elders with Mild Cognitive Impairment (MCI). Alpha power and peak parameters were computed in source space for a better spatial characterization of the alpha rhythm pattern in every group. With the specified sample groups we can assess and describe the changes and the evolution of the alpha power spectrum occurring along all the pre-dementia stages characterized to date.

3.4. Materials and methods

3.4.1. Subjects

The sample of this study was recruited from three different sources; the Neurology Department in "Hospital Universitario San Carlos", the "Center for Prevention of Cognitive Impairment" and the "Seniors Center of Chamartin District" located in Madrid (Spain). All participants were right-handed and native Spanish speakers.

The sample consisted of 131 elders. 51 of them were catalogued as mild cognitive impairment (MCI group), while 80 showed no objective neuropsychological impairment. The latter were further divided in two groups, 39 elders without any cognitive concern (no SCD group) and 41 with subjective cognitive decline (SCD group). Table 3-1 summarizes their demographic data and other relevant characteristics.

3.4.2. Diagnostic criteria

In order to assess the general cognitive and functioning status in the sample a set of screening questionnaires were administered to every participant: The Mini Mental State Examination (MMSE; (Lobo, Ezquerra, Gómez Burgada, Sala, & Seva Díaz, 1979)), the Geriatric Depression Scale – Short Form (GDS-SF; (Yesavage et al., 1982)), the Hachinski Ischemic Score (HIS; (Rosen, Terry, Fuld, Katzman, & Peck, 1980)) and the Functional

Assessment Questionnaire (FAQ; (Pfeffer, Kurosaki, Harrah, Chance, & Filos, 1982)).

After the initial screening all subjects underwent an exhaustive neuropsychological assessment including: Direct and Inverse Digit Span Test (Wechsler Memory Scale, WMS-III),Immediate and Delayed Recall (WMS-III), Phonemic and Semantic Fluency (Controlled oral Word Association Test, COWAT), Ideomotor Praxis of Barcelona Test, Boston Naming Test (BNT) and Trail Making Test A and B (TMTA and TMTB) (Reitan, 1958) and Rule Shift Cards (Behavioural Assessment of the Dysexecutive Syndrome, BADS).

Subjects were diagnosed as MCI according to the criteria established by Petersen (Petersen, 2004) and Grundman (Grundman, 2004). MCI patients did not fulfill criteria for dementia diagnosis.

	Mean ± SD			p-values		
	NoSCD	**SCD**	**MCI**	**NoSCD-SCD**	**NoSCD-MCI**	**SCD-MCI**
Age	70.4 ± 3.7	71.6 ± 4.5	73 ± 3.7	NS	0.005	NS
Gender (M-F)	12 - 27	9 - 32	22 - 29	-	-	-
Education (years)	14.5 ± 5.5	14.2 ± 5.8	9.5 ± 4.9	NS	$1.6{\cdot}10^{-4}$	$3.2{\cdot}10^{-4}$
GDS	0.9 ± 1.1	1.4 ± 1.2	2.7 ± 2.1	NS	$2.4{\cdot}10^{-6}$	$3.3{\cdot}10^{-6}$
FAQ	0 ± 0	0.1 ± 0.5	1.4 ± 1.7	NS	$1.8{\cdot}10^{-6}$	$6.3{\cdot}10^{-6}$
MMSE	29 ± 1.1	28.9 ± 1.1	27.4 ± 2	NS	$4.8{\cdot}10^{-5}$	$2.4{\cdot}10^{-6}$
Direct Digit	8.5 ± 1.9	8.8 ± 2.1	7.1 ± 2.1	NS	0.011	$6.5{\cdot}10^{-5}$
Inverse Digit	6.2 ± 2.1	5.7 ± 2	4.4 ± 1.5	NS	$7.5{\cdot}10^{-5}$	0.006
Inmediate recall	42.7 ± 11	36.2 ± 11.6	17.4 ± 9	0.032	$9.6{\cdot}10^{-10}$	$9.6{\cdot}10^{-10}$
Delayed recall	26.3 ± 8	21.2 ± 8.7	6.5 ± 7.8	0.032	$9.6{\cdot}10^{-10}$	$9.6{\cdot}10^{-10}$
Rule Shift Cards	3.5 ± 0.7	3.2 ± 1	2.5 ± 1.3	NS	0.002	0.038
FAS-phonemic	14.4 ± 4.7	12.2 ± 3.8	9.1 ± 4.1	NS	$1.6{\cdot}10^{-7}$	0.003
FAS-semantic	18.4 ± 3.4	17 ± 2.7	13.1 ± 3.5	NS	10-sep	$2.9{\cdot}10^{-7}$
TMTA (hits)	24 ± 0.2	23.9 ± 0.5	23.9 ± 1.1	-	-	-
TMTA (time)	48.2 ± 19.6	54.4 ± 22.1	77.1 ± 32.4	NS	$2.5{\cdot}10^{-5}$	$3.8{\cdot}10^{-4}$
TMTB (hits)	23.2 ± 3	22.1 ± 3.4	19.7 ± 6.1	NS	0.006	0.052
TMTB (time)	97 ± 43.5	126.5 ± 67.2	211.7 ± 101.5	NS	1.9·10-8	$8.1{\cdot}10^{-6}$
Idemotor praxis	7.8 ± 0.6	7.5 ± 0.9	7.1 ± 1.2	NS	0.045	NS
BNT	53 ± 8.7	50.9 ± 6.3	44.7 ± 8.6	NS	$5.9{\cdot}10^{-5}$	0.002
Hippocampal vol	$5{\cdot}10^{-3} \pm 5{\cdot}10^{-4}$	$5{\cdot}10^{-3} \pm 4{\cdot}10^{-3}$	$4{\cdot}10^{-3} \pm 7{\cdot}10^{-4}$	NS	$3.5{\cdot}10^{-4}$	$2.2{\cdot}10^{-4}$
IAF	9.8 ± 0.9	9.6 ± 0.9	9 ± 0.9	NS	$2.7{\cdot}10^{-4}$	0.010

Table 3-1. **Demographic, neuropsychological and neurophysiological data for each group: No-SCD (N = 39), SCD (N = 41) and MCI (N = 51).** The left part of the table shows mean ± standard deviation of the demographic and clinical measurements. The right

part shows ANOVA p-values after multiple comparisons when the p-value for the factor is lower than 0.05, otherwise a hyphen is shown; NS, p-value for the factor was greater than 0.05. GDS, Geriatric Depression Scale-Short Form; FAQ, Functional Activity Questionnaire; MMSE, Mini Mental State Examination; TMTA, Trail-Making Test part A; TMTB, Trail-Making Test part B; BNT, Boston Naming Test; IAF, Individual Alpha Frequency averaged over posterior sensors.

Cognitive concerns were self-reported by the participants in an interview with clinician experts. The final group assignment was made by multidisciplinary consensus (neuropsychologists, psychiatrists and neurologist) after neuropsychological evaluation. Possible confounders of SCD such as medication, psycho-affective disorders or other relevant medical conditions were dismissed by the clinicians. Following the recommendations made by the SCD-I-WG all subjects were older than 60 at onset of SCD and this occurred within the last 5 years.

The exclusion criteria employed in this study were the followings: 1) history of psychiatric or neurological disorders or drug consumption that could affect MEG activity such as cholinesterase inhibitors, 2) evidence of infection, infarction or focal lesions in a T2-weighted scan within 2 months before MEG acquisition 3) a modified Hachinski score equal to 5 or higher, 4) a GDS-SF score equal to 5 or higher, 5) alcoholism, chronic use of anxiolytics, neuroleptics, narcotics, anticonvulsants or sedative hypnotics. All participants were between 65 and 80 years old. Besides, we conducted additional analysis to rule out other possible causes of cognitive decline such as B12 vitamin deficit, diabetes mellitus, thyroid problems, syphilis, or Human Immunodeficiency Virus (HIV).

All participants signed an informed consent prior to study enrollment. This study was approved by the Clínico San Carlos Hospital ethics committee and the procedure was performed in accordance with approved guidelines and regulations.

3.4.3. MEG recordings

Neurophysiological data was acquired by using a 306 channel (102 magnetometers, 204 planar gradiometers) Vectorview MEG system (Elekta AB, Stockholm, Sweden), placed in a magnetically shielded room (VacuumSchmelze GmbH, Hanau, Germany) at the "Laboratory of Cognitive and Computational Neuroscience" (Madrid, Spain). All recordings were obtained in the morning, while subjects were sat comfortably, resting awake with eyes closed. Four minutes of MEG signal was acquired for each subject.

Head shape was obtained by using a three-dimensional Fastrak digitizer (Polhemus, Colchester, Vermont). Three fiducial points were acquired (nasion and left and right preauricular points) and at least 300 points of the

surface of the scalp. In addition, four head position indication (HPI) coils were placed on the subjects scalp, two in the mastoids and two in the forehead. HPI coils' position was also acquired using the Fastrak device, in order to provide continuous head position estimation during the recording. Finally, two vertical electrooculogram electrodes were placed above and beneath the left eye of the participants to capture eye blinks and movements.

MEG data was acquired using a sampling rate of 1000 Hz using an online anti-alias filter of [0.1 330] Hz. Recordings were filtered offline using a tempo-spatial filtering algorithm (tSSS, correlation window 0.9, time window 10 seconds) (Taulu & Simola, 2006) to eliminate magnetic noise originated outside the head and compensate for head movements

3.4.4. MRI acquisition

A T1-weighted MRI was available for each subject, acquired in a General Electric 1.5 Tesla magnetic resonance scanner, using a high-resolution antenna and a homogenization PURE filter (Fast Spoiled Gradient Echo sequence, TR/TE/TI = 11.2/4.2/450 ms; flip angle 12°; 1 mm slice thickness, 256x256 matrix and FOV 25 cm). Hippocampal volumes were measured as anatomical evidences of brain atrophy characteristic for MCI and AD (Dubois et al., 2007). MRI images were processed with Freesurfer software (version 5.1.0) and its specialized tool for automated cortical and subcortical segmentation (Fischl et al., 2002) in order to obtain the volume of several brain areas. Finally, hippocampal volume was normalized with respect to the overall intracranial volume (ICV) to account for differences in head volume over subjects.

3.4.5. MEG Signal preprocessing

Ocular, muscular and jump artifacts were first identified using an automatic procedure from the Fieldtrip package (Oostenveld, Fries, Maris, & Schoffelen, 2011), and then visually confirmed by a MEG expert. The remaining data was segmented in 4 seconds epochs of artifact-free activity. Only magnetometers data were used in the subsequent analysis. Subjects with at least 15 clean epochs were selected for further analysis (47.6 ± 7.3 epochs in the no SCD group, 46.2 ± 9.4 epochs in the SCD group, 42.2 ± 7.0 epochs in the MCI group, mean ± standard deviation). There was no significant group effect regarding the number of trials ($p=0.64$). In addition, an ICA-based procedure was employed to remove the electrocardiographic component.

3.4.6. Source reconstruction

Artifact-free epochs were pass-band filtered between 2 and 45 Hz, in order to remove both low frequency noise and network line artifact. The epochs

were padded with 2 seconds of real signal from both sides (2000 samples) prior to the filtering to prevent edge effects inside the data.

The source model consisted of 2459 sources placed in a homogeneous grid of 1cm in MNI template, then linearly transformed to subject space by warping the subject MRI into the MNI template . The leadfield was calculated using three-shell Boundary Element Method (using brain-skull, skull-scalp and scalp-air interfaces) generated from the T1 MRI using Fieldtrip package and OpenMEEG software (Gramfort, Papadopoulo, Olivi, & Clerc, 2010). Lastly, a Linearly Constrained Minimum Variance (LCMV) beamformer (Van Veen, van Drongelen, Yuchtman, & Suzuki, 1997) was employed to obtain the source time series by using the computed leadfield and building the beamforming filter with the epoch-averaged covariance matrix.

3.4.7. Spectral analysis

The estimated spatial filters were employed to reconstruct the source-space time series for each epoch and source location. MEG power spectra were calculated for every clean epoch with Fieldtrip toolbox. A multitaper method with discrete prolate spheroidal sequences as tapers and 0.5 Hz smoothing for frequencies between 2 and 45 Hz with 0.5 Hz steps was employed. Then the obtained power was normalized with the overall power in [2-45] Hz, thus obtaining the values of the relative power spectra for each frequency step, source and subject.

In order to calculate alpha band power in the most representable and comprehensive way for our sample we manually identified the individual alpha frequency (IAF) for every participant as the most prominent alpha peak in the average power spectrum over occipital and temporal channels. The average IAF in the study sample was 9.4 Hz. Then according to Klimesch considerations about alpha band width (Klimesch, 1999) we set it from 6.9 Hz to 11.4 Hz (i.e. IAF-2.5 Hz – IAF + 2 Hz). Alpha relative power for every source and subject was then computed as the sum of the relative power within the alpha range. Besides, we inspected any other possible power group level effect in theta [4 6.9 Hz] and beta [12 30 Hz] bands following the same procedure and no significant differences were found between groups.

Additionally, alpha peaks parameters were extracted by fitting the source power spectra $P(f)$ to a Gaussian peak with power law background, following (Chiang et al., 2008; Garcés et al., 2013).

$$\log\big(P(f)\big) = \mathrm{B} - \mathrm{C} \cdot \log(\mathrm{f}) + \mathrm{A} \cdot \exp\left(\frac{-(f-f_p)^2}{\Delta^2}\right) \qquad \textbf{(1)}$$

where A, B, C, Δ and f_p are fitted with a non-linear least square procedure. A wide fitting range of 4-14 Hz was set. Alpha peak frequencies f_p were selected for further analyses.

3.4.8. Statistical analysis

Statistical analyses in this work were performed in a multi-level approach. Differences across groups regarding neuropsychological scores, alpha relative power and alpha peak frequency were all assessed using an ANCOVA procedure with age as a covariate.

Tukey's HSD (Honestly Significant Difference) multiple comparisons correction was performed in those neuropsychological tests showing a significant between-groups effect in order to identify which groups' scores differed.

For source-space alpha peak power and frequency analyses Cluster Based Permutation Tests (CBPT) (Maris & Oostenveld, 2007) were employed for multiple comparisons corrections. The CBPT were executed with 2000 repetitions and a Montecarlo procedure, using always the age as covariate. Alpha level was set to 0.05.

Finally, to assess the structural and behavioural counterparts of the electrophysiological changes we conducted several linear regression models using power and peak frequency values for each subject as predictor variables to estimate neuropsychological performance and hippocampal volume. For these analyses alpha power and peak frequency were averaged across cortical sources. Both variables were included in a single step in the regression as there exists no theoretical definitive justification for ordering the entry of these predictor variables in the model. The equation describing the multiple linear regressions employed is:

$$y_i = \beta_0 + \beta_1 x_{i1} + \beta_2 x_{i2} + \varepsilon \qquad \textbf{(2)}$$

where y is the criterion variable (i.e. neuropsychological performance in each test or hippocampal volume), x_1 and x_2 are the variables employed to predict y (alpha relative power and frequency peak respectively), β_0 is the intercept term, β_1 and β_2 are the estimated coefficient for each predictor in the model and ε is the error in the prediction of y. Then a false discovery rate (FDR, (Benjamini & Hochberg, 1995) with q = 0.1 was employed to correct for multiple comparisons.

3.5. Results

3.5.1. Neuropsychological assessment

Neuropsychological performance along with group level differences is listed in Table 3-1. Of note, the performance of SCD subjects in every neuropsychological measurement laid within a 1SD distance from the average performance of no SCD group. This is crucial to verify their healthy state and rule out any possible objectifiable cognitive decline. Although SCD group scores in Immediate and Delayed Recall were significantly lower compared with no SCD group, each SCD participant performed in the normal range for those subtests. Secondly, MCI patients performed significantly worse than both no SCD and SCD groups in the vast majority of the tests employed which covered: working memory, language, executive functions and praxis.

3.5.2. Differences in hippocampal volume

Hippocampal volumes obtained with Freesurfer software were compared with a one-way ANCOVA (Table 3-1). Diagnosis served as the main factor while the age was included in the analysis as a covariate. The effect of diagnosis was significant in the specified contrast ($p<0.01$). Pair-wise comparisons revealed a reduction in hippocampal volume for MCI patients against both SCD ($p<0.01$) and no SCD groups ($p<0.01$). There were no differences in hippocampal volume between SCD and no SCD groups.

3.5.3. Differences in alpha peak frequency

Table 3-1 displays the average IAF per group. As above-mentioned we visually identified IAF values from the posterior sensors for each subject. ANCOVA revealed a significant effect of diagnosis ($p<0.01$). Pair-wise comparisons revealed no significant differences between no SCD and SCD groups, thus evidencing a normal alpha peak frequency in SCD participants. MCI subjects presented significantly lower IAF than both SCD and no SCD participants. To further analyze these results and determine the topology of the peak frequency change we performed a source–level comparison of IAF.

No SCD vs SCD

Source space comparison of the alpha peak frequency confirmed the previously reported results for the sensor space. No significant differences were found between these two groups.

No SCD vs MCI

There were significant differences between MCI patients and no SCD group regarding alpha peak frequency ($p<0.05$). Peak frequency was significantly

lower for MCI patients over several areas encompassing bilateral temporal structures such as hippocampus and parahippocampal cortices and both temporal poles, parietal areas including bilateral precentral, postcentral and supramarginal gyri and also bilateral frontal areas (Figure 3-1).

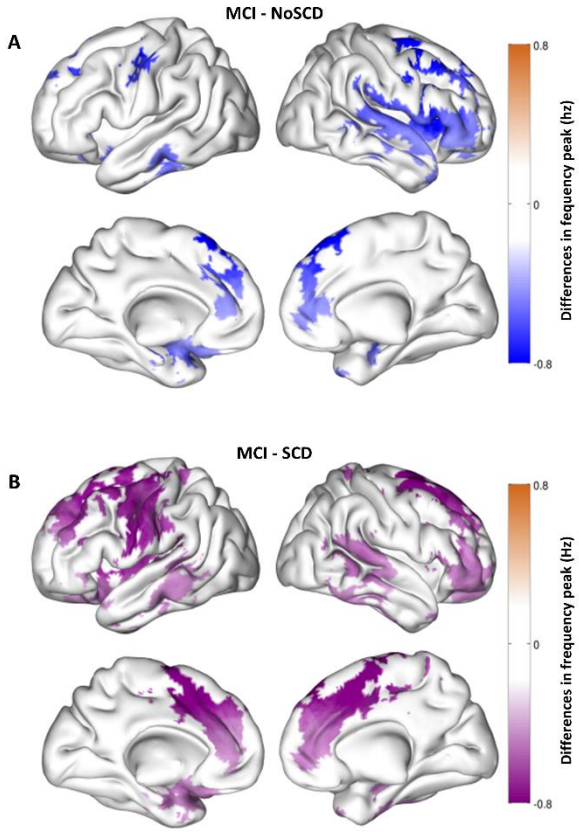

Figure 3-1. This figure displays the significant differences in the frequency of the alpha peak between groups in the source space.

A (top): Differences between MCI and no SCD. Blue areas represent higher frequency of the alpha peak power in the no SCD group.
B (bottom): Differences between MCI and SCD. Purple areas represent higher frequency of the alpha peak in the SCD group.

SCD vs MCI

Statistical testing revealed a slowing in MCI alpha rhythm when compared with the SCD group ($p<0.05$). Interestingly, MCI slowing for this contrast exhibited a very similar distribution to the one found for the MCI vs no

SCD comparison. Areas with larger differences included bilateral medial temporal structures and bilateral parietal and frontal areas (Figure 3-1).

3.5.4. Differences in alpha relative power

No SCD vs SCD

Significant differences were found in alpha relative power between no SCD and SCD subjects ($p<0.01$). SCD elders exhibited decreased alpha relative power over wide brain regions. The strongest differences were located over bilateral prefrontal areas, bilateral middle and superior temporal lobe, and also bilaterally over calcarine fissure and cuneus in the occipital lobe (Figure 3-2).

No SCD vs MCI

Alpha band relative power was significantly diminished in MCI patients when compared with healthy elders without SCD ($p<0.05$). These differences were more evident over bilateral occipital areas (Inferior occipital, calcarine, lingual gyrus and cuneus), bilateral prefrontal areas (orbitofrontal cortex, middle and superior frontal gyri) and small portions of the bilateral temporal poles (Figure 3-2).

SCD vs MCI

There were no significant differences between SCD participants and MCI group in alpha power after multiple comparisons correction. Thus both groups exhibited a decrease in alpha power with respect to no SCD group with no differences between them in that regard.

3.5.5. Multiple linear regressions

We performed several multiple linear regressions, using as regressed variable each of the 14 neuropsychological tests and hippocampal volume. The complete set of results is listed in Table 3-2. After FDR alpha relative power accounted for a significant proportion of the variance in the execution of the following tests: Inverse Digits, Rule Shift Cards, TMTB (both for hits and time) and BNT. The estimated coefficient predicted a better performance in the test with an increase in alpha relative power for all of them. There was a trend toward significance in the same direction for TMTA (time) ($p=0.06$). Regarding alpha peak frequency, it explained a significant amount of variance in 11 different models: MMSE, Immediate and Delayed Recall, FAS Semantic, TMTA (both for hits and time), TMTB (hits), Ideomotor Praxis and BNT and hippocampal volume and a trend toward significance for TMTB (time) ($p=0.08$). All of the coefficients pointed to a consistent improvement in the execution (i.e. better performance and lower time in TMT) or a higher hippocampal volume with an increase in the frequency of the alpha peak.

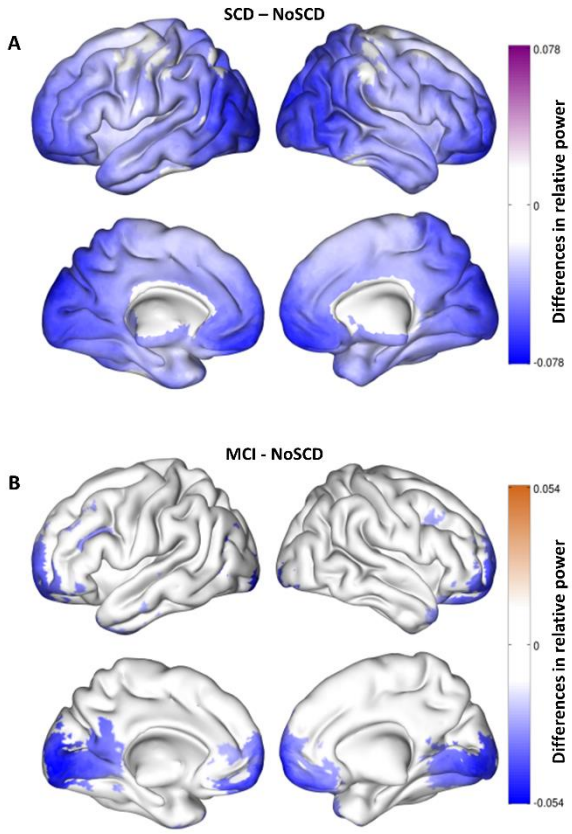

Figure 3-2. This figure displays the significant differences in alpha relative power between groups in the source space.

A (top): Differences between SCD and no SCD. Blue areas represent higher alpha relative power in the no SCD group.
B (bottom): Differences between MCI and no SCD. Blue areas represent higher alpha relative power in the no SCD group.

	Estimate ± SE		p-values	
Criterion	**Alpha power**	**Peak frequency**	**Alpha power**	**Peak frequency**
MMSE	-0.71	0.47	NS	0.015
Direct Digit	1.63	0.18	NS	NS
Inverse Digit	7.28	0.34	0.032	NS
Inmediate recall	34.67	4.29	NS	0.016
Delayed recall	25.99	3.81	NS	0.006
Rule Shift Cards	4.10	0.42	0.044	0.006
FAS-phonemic	7.99	0.32	NS	NS
FAS-semantic	11.83	1.09	NS	0.019
TMTA (hits)	0.44	0.22	NS	0.016
TMTA (time)	-90.72	-8.72	NS	0.010
TMTB (hits)	18.56	2.02	0.019	2.9×10^{-4}
TMTB (time)	-326.4	-19.4	0.043	NS
Ideomotor praxis	2.35	0.30	NS	0.009
BNT	28.86	2.41	0.052	0.019
Hippocampal vol	-1.1×10^{-3}	2.2×10^{-4}	NS	0.008

Table 3-2. **Multiple linear regression models for a set of neuropsychological and neurophysiological measurements.** Each row shows the results for a regression model using the criterion as the predicted variable and alpha power and the frequency of the peak as the predictor variables. Coefficients for each predictor are listed. The right part shows p values for each significant coefficient and 'NS' stands for not significant after FDR (q=0.1); MMSE, Mini Mental State Examination; TMTA, Trail-Making Test part A; TMTB, Trail-Making Test part B; BNT, Boston Naming Test.

3.6. Discussion

The present study seeks to determine whether oscillatory alpha activity measured with MEG sheds light on the discussed consideration of the SCD stage as a part of the AD-continuum. Our results certainly show alterations in the alpha band of SCD participants consistent with AD. Both SCD and MCI groups show common features in their electrophysiological profile. One of the main findings of this study is the reduction in alpha relative power in healthy elders presenting subjective cognitive decline across broad brain regions when compared with age and sex matched counterparts without any cognitive concern. This reduction in alpha relative power was also present in the MCI group. Furthermore, SCD patients did not show any sign of alpha peak frequency slowing, while MCI patients did. In addition, these reductions in alpha power and especially in peak frequency were significantly related with a worse performance in many neuropsychological tests, which confirms their pathological nature. These findings support the interpretation of SCD as the first recognizable stage to date in the AD spectrum and a key phase in the early detection of the Alzheimer´s Disease.

A decrease in MEG power is closely connected with a reduction in the number of active synchronous synapses in a specific frequency range (Buzsáki, 2006), as MEG signals derive from the spatio-temporal summation of postsynaptic potentials. A plausible hypothesis for this alpha power reduction in SCD participants is that a certain degree of synaptic damage is already present. This would be consistent with the fact that Aβ levels may increase in the brain decades before any plaque deposition or cognitive symptom appears (Jack et al., 2010). Furthermore, Aβ has been shown to induce neuronal death through the activation of astrocytes with a potential mechanism involving nitric oxide (Hu, Ferreira, & Van Eldik, 2002) and also through neuro-inflammatory responses (Kitazawa, Yamasaki, & LaFerla, 2004). In fact, amyloid-β deposition correlates with cortical atrophy over broad brain areas including hippocampus, medial frontal, parietal areas, and lateral temporoparietal cortex in elderly population presenting SCD (Chételat et al., 2010). These findings support the widespread distribution of the alpha power decrement found in SCD in our work. In addition, elders with SCD accumulated more Aβ over frontal areas as reported in recent PET-PIB studies (Snitz et al., 2015). In the present study SCD participants exhibited largest power reduction over frontal areas, which is in agreement with the above mentioned studies. FDG-PET studies also reinforce this early synaptic damage hypothesis as elders with SCD were found to exhibit hypometabolism in AD typical areas (for a review see, [53]). Regarding electrophysiological studies in SCD, to the best of our knowledge the only study to date reporting spectral power resting state changes in SCD reported an overall increase in absolute alpha power, especially over frontal sensors (Alexander et al., 2006). However, their results cannot be directly compared with the present work given that authors did not normalize alpha power and especially due to discrepancies in sample recruitment. While in our study SCD subjects showed no cognitive impairment, in the above commented study SCD group performance was apparently affected in every cognitive domain they examined, which makes their sample barely comparable with ours and less representative of the SCD stage.

MCI patients also showed a decrease in alpha relative power compared with healthy elders without subjective cognitive decline in our sample. Reduction in alpha band power in MCI has been largely reported in the literature as one of the most robust and consistent changes in the MCI power spectrum (Babiloni et al., 2009; López et al., 2014). However the present work adds valuable spatial information as MEG data was reconstructed in the source space.

SCD and MCI patients showed a comparable decrease in alpha relative power as no significant differences emerged from this comparison. However, SCD´s power reduction seemed to affect broader brain regions

than the decrease exhibited by MCI group. A possible explanation for this finding might be related to the 1/f shape of the human power spectrum (i.e. power increases as the frequency decreases). This fact along with the alpha frequency slowing in MCI patients could attenuate the differences in alpha power between MCI and no SCD participants, as MCI alpha peak is localized in a slightly lower frequency range. The fact that the areas showing largest alpha decrease in SCD participants (i.e. frontal and occipital areas) were similar to those where MCI exhibited alpha relative power reduction seems to support this explanation.

SCD group did not show any slowing of their alpha peak frequency. Furthermore, both SCD and no SCD elders showed a significant higher alpha peak frequency compared to MCI patients. Previous studies have reported this slowing pattern for MCI (Garcés et al., 2013) and AD both compared to healthy controls (Passero, Rocchi, Vatti, Burgalassi, & Battistini, 1995). However, as far as we know this is the first study addressing the exact frequency of the peak for multiple brain regions in SCD elders. Furthermore, brain areas affected by this slowing in MCI patients were almost exactly the same in the two comparisons, against both SCD and no SCD. It is worth noting that the regions showing substantial slowing highly overlapped with Default Mode Network (DMN) areas: these included medial prefrontal areas, lateral temporal cortex, hippocampus formation and parietal areas. Disruption of the DMN has been previously reported with MEG, and it has been proven to relate to structural damage in white matter tracts in MCI patients (Garcés et al., 2014). While it is important to point out that our analysis does not capture long range connectivity, our results show a significant decrease of the alpha peak frequency in areas closely linked to the DMN, evidencing in these same regions some degree of synaptic disruption. Cortico-thalamo-cortical models have been employed to simulate changes in the alpha band. (Hindriks & van Putten, 2013) reported that a decrease in excitatory firing rate and an increase in inhibitory firing rate produced a slowing of the alpha peak. Alpha peak slowing is also related to a reduction in the number of active synapses in the thalamic nuclei (Bhattacharya, Coyle, & Maguire, 2011). This could suggest a significant synaptic damage in the MCI stage, for which an alpha slowing has been found.

Although exhibiting a pronounced reduction in alpha relative power, elders with subjective cognitive decline did not show evidences of slowing in their alpha peak, thus being comparable to healthy elders without cognitive concerns in this regard. These results altogether seem to draw a continuum in the AD with respect to alpha band changes. Accordingly, the first sign of the AD pathology in the spectral profile would be a reduction in alpha relative power, which might be related, as mentioned earlier to some degree of synaptic dysfunction. It would be later in the AD-continuum, in the MCI

stage, where the slowing of the power spectrum would take place. Several studies have revealed that axonal damage markers are already present in the MCI stage (Garcés et al., 2014; Zetterberg et al., 2016). According to Buzsaky [47] this impairment in axon conductance among other parameters, limits the capability of neurons to oscillate in higher frequency rhythms, which could in part explain the slowing we found in the MCI group. Furthermore, this progressive pattern of disruption in the alpha band conforms to thalamo-cortico-thalamic network simulations. Variation of synaptic connections in the thalamic module shows limited effect on the dominant frequency of the alpha band but systematically affect alpha power (Bhattacharya et al., 2011). In addition, these simulations showed that changes in alpha frequency are much smaller than changes in alpha amplitude (Hindriks & van Putten, 2013), which could make them undetectable in the very early stages of AD.

We studied how alpha band disruption is associated with cognitive changes in our sample. As we expected, both the decrease in alpha relative power, and the slowing of the peak predicted a worse performance in a variety of cognitive domains such as working memory, executive functions, praxis and language. Interestingly, the frequency of the peak demonstrated higher predictive power in multiple tests. This might be due to the fact that changes in alpha power appear in the SCD stage, where some kind of compensation could be still present, as no neuropsychological deficits can be observed. Therefore even though alpha power showed a significant association with performance, the slowing of the peak seems to be intimately related with cognitive decline, as both symptoms appear in the MCI stage.

Hippocampal volume comparisons only revealed a significant reduction in the MCI patients. There are two alternative interpretations of this result. On the one hand, low sensitivity of the automatic segmentation procedures on 1,5 T MRI images to detect hippocampal reduction in SCD has been recently highlighted (Perrotin et al., 2015). This study reported hippocampal reduction in SCD with a 3T system and manual segmentation. On the other hand, there are also studies reporting no hippocampal reduction in SCD (Jessen et al., 2006). An alternative interpretation is that MEG is able to detect functional alterations in the brain network before structural damage takes place. A 93.3% sensitivity predicting MCI converters has been achieved using spectral analysis of MEG data (Fernández, Gil Gregorio, & Maestú, 2012), underscoring MEG capability of detecting early signs of AD pathology in the brain.

An ever-increasing number of studies point out that SCD is the first identifiable stage of the AD-continuum. In this line, the alpha power reduction could constitute a marker of early AD pathology in the SCD stage which would be followed by a decrease in the frequency of alpha peak in

latter stages. These findings have an important clinical meaning since they point out an early synaptic dysfunction in the absence of a sensitive neuropsychological parameter. Thus, a non-invasive technique as MEG could be a good biomarker to detect early neuropathology in the AD-continuum. However, further research is required to confirm this pattern and more importantly to specify and concretize the optimal definition of SCD to reduce inconsistency in the literature.

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Author contributions

DLS, NS, ICRR, MLD, MM, RL, MY, FM designed and conducted research, DLS, PG, RB, CC analyzed data, DLS wrote the manuscript, DLS and PG prepared figures. All authors reviewed the manuscript.

Additional information

The authors declare no competing financial interests.

3.7. Summary of conclusions

1. Alpha band activity was found in a lower frequency range than the 8-12 Hz classically used in electrophysiology. This finding of slower average alpha frequency is consistent with previous literature reporting lower individual alpha frequency in aged population.

2. SCD and MCI elders both showed significant alterations in their power spectra profile. This disruption was focused in the alpha band. SCD elders showed a highly significant decrease of their relative alpha power over widespread regions without any slowing in their alpha frequency with respect to healthy controls (HC). MCI group showed no differences in alpha relative power with respect to the SCD group while they also exhibited reduced alpha relative power localized over occipital and frontal regions. Additionally, MCI patients showed a significant slowing of their individual alpha frequency over several brain regions compared to both HC and SCD.

3. This experiment concluded that SCD alterations certainly resemble one of the electrophysiological hallmarks of AD (alpha power decrease) and thus, when considering this and previous evidence altogether, it seems plausible that SCD could represent an asymptomatic stage of AD.

4. Changes found in both SCD and MCI suggest a somewhat linear trajectory in the spectral profile deterioration from HC to MCI stage. The results suggest that there is an initial loss of local synchrony in the alpha frequency range leading to a power decrease at very early stages of the disease. This would eventually lead to alpha peak slowing with increasing AD pathology towards the MCI stage.

5. The present work confirmed previous findings and a significant deterioration of gray matter integrity was observed in the MCI group. More concretely, MCI patients´ hippocampal volume was significantly diminished with respect to both HC and SCD. Interestingly, SCD elders did not show any significant deterioration in this medial temporal region.

6. Synaptic dysfunction seems to be an early sign of AD pathology. Remarkably, MEG is a well-suited technique to capture this electrophysiological malfunctioning in the very early stages of the disease. Using MEG we were able to detect small alterations in the

SCD group´s power spectrum that were not captured by MRI volumetry over one of the key structures affected by early AD such as hippocampus.

7. The changes observed in alpha relative power were significantly related to cognitive performance as alpha relative power predicted in a set of linear regression models worse performance in various cognitive domains such as memory, executive functioning or language. Even more importantly, the individual alpha frequency showed stronger associations with cognitive performance in almost each cognitive domain. This latter result is highly consistent with our expectations, and suggests that alpha slowing could represent the event triggering a marked cognitive deterioration in the AD continuum, leading to the MCI stage once it reaches a certain level.

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4. Experiment 2

Connectivity analysis in SCD and MCI

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Functional connectivity disruption in Subjective Cognitive Decline and Mild Cognitive Impairment: a common pattern of alterations.

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4.1. Summary of objectives

1. AD is considered as a disconnection syndrome in which FC is heavily affected. The main objective of the present experiment is to assess whether cognitively normal elders with SCD present alterations in whole brain connectivity analysis.

2. This experiment also seeks to replicate previous findings in AD literature reporting impaired functional connectivity among different brain regions in MCI patients.

3. Connectivity alterations have been reported in relation to a number of different clinical manifestations. However, the specific objective of this chapter is to evaluate whether hypothetical FC alterations in the SCD group show any spatial overlap with those of MCI patients.

4. A cascading network failure has been recently proposed to occur throughout the AD continuum. However, the specific AD stages at which these changes take place has not been addressed so far. This experiment aims to determine if SCD elders already present initial signs of this cascading network failure. Additionally, the evolution of this progressive deterioration between SCD and MCI will be also studied.

5. AD is known to affect specific RSN as shown in multiple fMRI studies. Although there are some previous MEG studies addressing this issue in AD, the electrophysiological integrity of these networks has been scarcely studied. We aimed to determine whether preclinical AD affected these networks. More concretely, we compared FC over DMN and dorsal attentional network (DAN) in HC, SCD and MCI to study at which stage these RSN become impaired.

6. DMN is no longer considered as a unitary network and recent literature suggested that it may be formed by several subcomponents. One of our objectives was to identify if posterior and/or anterior parts of the DMN are impaired in the SCD and MCI stage. Additionally, we wanted to investigate whether these hypothetical alterations followed a similar trajectory in both subcomponents.

7. From a neuropsychological perspective one of the main interests of this experiment is to assess the relationship between FC alterations in the preclinical stages of AD and cognitive performance. This

study explored the possible mechanisms underlying cognitive decline in the very early stages of AD in relation with brain FC.

8. This study intended to explore the possible pathological nature of FC alterations in SCD and MCI. To this aim we conducted correlation analysis between FC values and hippocampal volume, which represents a sign of AD pathological burden in early and late stages.

4.2. Abstract

Functional connectivity (FC) alterations represent a key feature in Alzheimer´s Disease (AD) and provide a useful tool to characterize and predict the course of the disease. Those alterations have been also described in Mild Cognitive Impairment (MCI), a prodromal stage of AD. There is a growing interest in detecting AD pathology in the brain in the very early stages of the disorder. Subjective Cognitive Decline (SCD) could represent a preclinical asymptomatic stage of AD but very little is known about this population. In the present work we assessed whether FC disruptions are already present in this stage, and if they share any spatial distribution properties with MCI alterations (a condition known to be highly related to AD). To this end, we measured electromagnetic spontaneous activity with MEG in 39 healthy control elders, 41 elders with SCD and 51 MCI patients.

The results showed FC alterations in both SCD and MCI compared to the healthy control group. Interestingly, both groups exhibited a very similar spatial pattern of altered links: a hyper-synchronized anterior network and a posterior network characterized by a decrease in FC. This decrease was more pronounced in the MCI group.

These results highlight that elders with SCD present FC alterations. More importantly, those disruptions affected AD typically related areas and showed great overlap with the alterations exhibited by MCI patients. These results support the consideration of SCD as a preclinical stage of AD and may indicate that FC alterations appear very early in the course of the disease.

4.3. Introduction

The increase in life expectancy during the last decades has an inherent downside: the increase of dementia cases in the population (Brookmeyer, Johnson, Ziegler-Graham, & Arrighi, 2007). AD is the most common cause of dementia, accounting for approximately 60 to 80% of cases (Barker et al., 2002; Wilson et al., 2012), therefore its early diagnose has become a relevant public health issue. Early interventions at an early stage of the disease before the appearance of extensive brain lesions are the most promising approach for reducing the burden of dementia in the population (Imtiaz, Tolppanen, Kivipelto, & Soininen, 2014).

The description of Mild Cognitive Impairment (MCI), an intermediate state between healthy individuals and dementia patients, represented an important step towards the early diagnose of AD. MCI is characterized by slight cognitive impairment in one or more domains and increased risk of developing dementia (R C Petersen, 2004). The presence of AD-pathology in MCI patients is a very consistent finding (Tabatabaei-Jafari, Shaw, & Cherbuin, 2015; Villemagne & Chételat, 2016), and the annual conversion rate from MCI to AD has been estimated around 8% to 15% (Ronald C Petersen, 2016). Over the last few years there is a growing interest in what has been suggested to be a pre-clinical asymptomatic stage of AD: Subjective Cognitive Decline (SCD) (Jessen et al., 2014). Accordingly, SCD could represent a prodromal stage of MCI in which individuals report a worsening in their cognitive skills that current neuropsychological assessment tools are not able to capture. SCD subjects are at a greater risk for developing MCI or AD compared to healthy elders (Jessen et al., 2010, 2014; Mitchell, Beaumont, Ferguson, Yadegarfar, & Stubbs, 2014; Reisberg, Shulman, Torossian, Leng, & Zhu, 2010). Whilst there are still inconsistent results, some neuroimaging studies reported AD-related brain pathology in SCD supporting its interpretation as an asymptomatic stage of AD as reviewed by Sun, Yang, Lin, & Han (2015).

Among the variety of neuroimaging tools available, the International Working Group has recently highlighted the great sensitivity of functional connectivity (FC) EEG/MEG measures in the very early stages of AD, and the limited number of electrophysiological studies on this stage (Dubois et al., 2016). FC disruption in AD is a consistent finding in the literature (Gili et al., 2011; Teipel et al., 2016). In fact AD has been described as a disconnection syndrome (Delbeuck, Linden, & Collette, 2003). Moreover, these abnormalities have also been reported for MCI patients (Bai et al., 2009; Sorg et al., 2007) and have demonstrated great utility in predicting future cognitive decline and conversion to AD (López et al., 2014; Petrella, Sheldon, Prince, Calhoun, & Doraiswamy, 2011). However, the knowledge about the course of FC alterations in the very early stages of AD is really

scarce, and the few available results in fMRI resting state are inconsistent (Hafkemeijer et al., 2013; Wang et al., 2013). The only study to date using MEG in SCD reported a decrease in FC during the performance of a memory task (Bajo et al., 2012), but resting state FC was not addressed.

In this study, we reconstructed for the first time source space whole brain resting state functional networks with MEG of healthy elders, SCD and MCI patients in the alpha band. A previous study from out group demonstrated that SCD elders exhibit spectral alterations specific to this frequency range (López-Sanz et al., 2016). Furthermore, results in this frequency range have demonstrated great consistency in previous works studying MCI and AD patients (Babiloni et al., 2006) (Adler, Brassen, & Jajcevic, 2003; Jelic et al., 2000). Most of the studies reported a prominent decrease in FC in both MCI and AD patients. The study of other frequency bands reached somewhat inconsistent results (for a review, see Babiloni et al., 2015). Hence, we hypothesized that the onset of the FC cascading failure in AD takes place in the SCD stage, thus SCD patients will show an intermediate FC pattern of alterations between those exhibited by controls and MCI sharing some spatial properties. Additionally, in order to help the interpretation and comparison of our results with previous literature we conducted FC analysis in two key resting state networks (RSN) consistently associated with AD: default mode network (DMN) and dorsal attention network (DAN) (Greicius, Srivastava, Reiss, & Menon, 2004; Li et al., 2012).

4.4. Materials and methods

4.4.1. Subjects

The study sample was recruited from three different centers: the Neurology Department in "Hospital Universitario San Carlos", the "Center for Prevention of Cognitive Impairment" and the "Seniors Center of Chamartin District" located in Madrid (Spain).

The sample consisted of 131 right-handed and native Spanish speakers. 51 of them were diagnosed as mild cognitive impaired (MCI group), while 80 showed no objective neuropsychological impairment. The latter were further divided in two groups, 39 healthy control elders without any cognitive concern (HC group) and 41 with subjective cognitive decline (SCD group). Table 4-1 summarizes their demographic data and other relevant characteristics.

	Mean ± SD			*p*-values		
	Control	SCD	MCI	HC - SCD	HC - MCI	SCD - MCI
Age	70.4 ± 3.7	71.6 ± 4.5	73.0 ± 3.7	n.s.	$5.3 \cdot 10^{-3}$	n.s.
Gender (M/F)	1.7 ± 0.5	1.8 ± 0.4	1.6 ± 0.5	n.s.	n.s.	n.s.
GDS	0.9 ± 1.1	1.4 ± 1.2	2.7 ± 2.1	n.s.	$5.9 \cdot 10^{-7}$	$2.3 \cdot 10^{-4}$
MMSE	29.0 ± 1.1	28.9 ± 1.1	27.4 ± 2.0	n.s.	$1.7 \cdot 10^{-6}$	$5.8 \cdot 10^{-6}$
Direct digits	8.5 ± 1.9	8.8 ± 2.1	7.1 ± 2.1	n.s.	$2.8 \cdot 10^{-3}$	$2.5 \cdot 10^{-4}$
Inverse digits	6.2 ± 2.1	5.7 ± 2.0	4.4 ± 1.5	n.s.	$1.1 \cdot 10^{-5}$	$3.0 \cdot 10^{-3}$
BNT	53.0 ± 8.7	50.9 ± 6.3	44.7 ± 8.6	n.s.	$4.7 \cdot 10^{-6}$	$7.9 \cdot 10^{-4}$
Hippocampal volume	$5.0 \cdot 10^{-3} \pm 0.5 \cdot 10^{-3}$	$5.0 \cdot 10^{-3} \pm 0.7 \cdot 10^{-3}$	$4.4 \cdot 10^{-3} \pm 0.7 \cdot 10^{-3}$	n.s.	$5.8 \cdot 10^{-6}$	$4.0 \cdot 10^{-5}$

Table 4-1. **Demographic, neuropsychological and neurophysiological data for each group.** The left half of the table shows mean ± SD (standard deviation) of demographic information, some neuropsychological scores and neurophysiological data for each group. The right half of the table shows the p-values resulting of the ANOVA comparisons between groups. If the comparison is not significant ($p > 0.05$) the value is replaced by n.s. (non-significant). GDS stands for Geriatric Depression Scale - Short Form. MMSE stands for Mini Mental State Examination. BNT stands for Boston Naming Test.

4.4.2. Diagnostic criteria

To assess the general cognitive and functional status the following set of screening questionnaires were used: The Mini Mental State Examination (MMSE; (Lobo, Ezquerra, Gómez Burgada, Sala, & Seva Díaz, 1979)), the Geriatric Depression Scale–Short Form (GDS) (Yesavage et al., 1982); the Hachinski Ischemic Score, (HIS) (Rosen, Terry, Fuld, Katzman, & Peck, 1980)) and the Functional Assessment Questionnaire (FAQ); (Pfeffer, Kurosaki, Harrah, Chance, & Filos, 1982).

After the initial screening all subjects underwent an exhaustive neuropsychological assessment including: Direct and Inverse Digit Span Test (Wechsler Memory Scale, WMS-III), Immediate and Delayed Recall

(WMS-III), Phonemic and Semantic Fluency (Controlled oral Word Association Test, COWAT), Ideomotor Praxis of Barcelona Test, Boston Naming Test (BNT), Trail Making Test A and B (TMTA and TMTB) (Reitan, 1958) and Rule Shift Cards (Behavioural Assessment of the Dysexecutive Syndrome, BADS).

MCI group subjects were diagnosed as MCI according to the criteria established by Petersen (R C Petersen, 2004) and Grundman (Grundman, 2004). MCI patients did not fulfill criteria for dementia diagnosis.

Cognitive concerns were self-reported by the participants in an interview with clinician experts. The final group assignment was made after neuropsychological evaluation attending to a multidisciplinary consensus (by neuropsychologists, psychiatrists and neurologists). In order to prevent possible confounders of SCD, problematic medication, psycho-affective disorders or other relevant medical condition lead to the exclusion from the study. Following the recommendations made by the SCD-I-WG, all subjects were older than 60 at onset of SCD, and the onset of SCD occurred within the last 5 years.

The exclusion criteria employed in this study were the followings: 1) history of psychiatric or neurological disorders or drug consumption that could affect MEG activity, such as cholinesterase inhibitors; 2) evidence of infection, infarction or focal lesions in a T2-weighted scan within 2 months before MEG acquisition; 3) a modified Hachinski score equal or higher to 5; 4) a GDS-SF score equal to or higher to 5; 5) history of alcoholism, chronic use of anxiolytics, neuroleptics, narcotics, anticonvulsants or sedative hypnotics. All participants were between 65 and 80 years old at the moment of the MEG acquisition. Besides, additional analyses were conducted to rule out other possible causes of cognitive decline such as B12 vitamin deficit, diabetes mellitus, thyroid problems, syphilis, or Human Immunodeficiency Virus (HIV).

All participants signed an informed consent prior to study enrollment. This study was approved by the “Hospital Universitario San Carlos” ethics committee, and the procedures were performed in accordance with approved guidelines and regulations.

4.4.3. MEG recordings

Neurophysiological data was acquired by using a 306 channel (102 magnetometers, 204 planar gradiometers) Vectorview MEG system (Elekta AB, Stockholm, Sweden), placed inside a magnetically shielded room (VacuumSchmelze GmbH, Hanau, Germany) at the “Laboratory of Cognitive and Computational Neuroscience” (Madrid, Spain). All recordings were obtained while subjects were sitting comfortably, resting

awake with eyes closed. MEG acquisition consisted of four minutes of signal for each subject.

Head shape was obtained by using a three-dimensional Fastrak digitizer (Polhemus, Colchester, Vermont). Three fiducial points (nasion and left and right preauricular points) and at least 300 points of the surface of the scalp were acquired for each subject. In addition, four head position indication (HPI) coils were placed on the subjects scalp, two in the mastoids and two in the forehead. HPI coils' position was also acquired using the Fastrak device, and continuous head position estimation was used during the recording in order to track head movements. Finally, a vertical electrooculogram of the left eye was used to capture blinks and eye movements.

MEG data was acquired using a sampling rate of 1000 Hz and an online anti-alias bandpass filter between 0.1 and 330 Hz. Recordings were processed offline using a spatiotemporal signal space separation algorithm (Taulu & Simola, 2006) (correlation window 0.9, time window 10 seconds) in order to remove magnetic noise originated outside the head. The algorithm was also used to correct head movements of the subject during the recording.

4.4.4. MEG Signal preprocessing

Ocular, muscular and jump artefacts were first identified using an automatic procedure from the Fieldtrip package (Oostenveld, Fries, Maris, & Schoffelen, 2011), and then visually confirmed by a MEG expert. The remaining data was segmented in 4 seconds epochs of artefact-free activity. Subjects with at least 15 clean epochs were selected for further analysis (47.6 ± 7.3 epochs in the HC group, 46.2 ± 9.4 epochs in the SCD group, 42.2 ± 7.0 epochs in the MCI group, mean ± standard deviation). In addition, an ICA-based procedure was employed to remove the electrocardiographic component when it was clearly identified. Due to data redundancy after the spatiotemporal filtering, only magnetometers data were used in the subsequent analysis.

4.4.5. MRI acquisition

A T1-weighted MRI was available for each subject, acquired in a General Electric 1.5 Tesla magnetic resonance scanner, using a high-resolution antenna and a homogenization PURE filter (Fast Spoiled Gradient Echo sequence, TR/TE/TI = 11.2/4.2/450 ms; flip angle 12°; 1 mm slice thickness, 256x256 matrix and FOV 25 cm). MRI images were processed with Freesurfer software (version 5.1.0) and its specialized tool for automated cortical and subcortical segmentation (Fischl et al., 2002) in order to obtain the volume of several brain areas. Hippocampal volumes

were selected as anatomical evidences of brain atrophy characteristic for MCI and AD (Dubois et al., 2007), and normalized with respect to the overall intracranial volume (ICV) to account for differences in head volume over subjects.

4.4.6. Source reconstruction

The source model consisted of 2459 sources placed in a homogeneous grid of 1cm in MNI template, then linearly transformed to subject space. Each source was labelled as belonging to one of the 64 areas of the reduced Harvard-Oxford atlas (Desikan et al., 2006). Of the initial 2459 sources, 970 were discarded due to not being identified as part of any recognized area (i.e. white matter sources). The leadfield was calculated for the remaining 1489 sources using a three-shell Boundary Element Method (brain-skull, skull-scalp and scalp-air interfaces generated from the subject's T1 MRI) model computed with OpenMEEG software (Gramfort, Papadopoulo, Olivi, & Clerc, 2010).

Source reconstruction was performed for the alpha band. In order to calculate alpha band in the most representable and comprehensive way for our sample we visually identified the individual alpha frequency (IAF) for every participant as the most prominent alpha peak in the average power spectrum over occipital and parietal channels. The average IAF in the study sample was 9.4 Hz. Then, according to Klimesch considerations about alpha band width (Klimesch, 1999), we set it from 6.9 Hz to 11.4 Hz (i.e. IAF-2.5 Hz – IAF + 2 Hz).

Artefact-free epochs were bandpass filtered in the alpha band using an 1800 order FIR filter designed using Hanning window. Data was filtered in a two-pass procedure to avoid phase distortion, and using 2000 samples of data at each side as padding to avoid edge effects. Lastly, a Linearly Constrained Minimum Variance (LCMV) beamformer (Van Veen, van Drongelen, Yuchtman, & Suzuki, 1997) was employed to obtain the source time series by using the computed leadfield and the epoch-averaged covariance matrix.

4.4.7. Connectivity analysis

The analysis of FC was performed using the hypothesis of phase synchronization (Rosenblum, Pikovsky, & Kurths, 2001) and evaluated using the Phase Locking Value (PLV) (Lachaux, Rodriguez, Martinerie, & Varela, 1999). PLV is calculated using the instantaneous phase difference between a pair of signals. For each temporal point, a vector is constructed with norm unity and phase equal to the phase difference between the instantaneous phases of both signals. Then, PLV value for each data

segment is calculated as the norm of the average vector for the pair of signals k and l:

$$PLV_{k,l} = \left| \frac{1}{T} \sum_{t} e^{-j(\varphi_k(t) - \varphi_l(t))} \right|$$

where $\varphi_k(t)$ is the instantaneous phase of signal k at instant t, and T the number of temporal points per segment.

In order to reduce the dimensionality of the connectivity matrices, PLV values are averaged over areas, obtaining a unique PLV value for each pair of areas defined in the reduced Harvard-Oxford atlas. The final PLV value between areas A and B is calculated as follows:

$$PLV_{A,B} = \frac{1}{N_A N_B} \sum_{N_A} \sum_{N_B} \left| \frac{1}{T} \sum_{t} e^{-j\left(\varphi_{A_k}(t) - \varphi_{B_l}(t)\right)} \right|$$

where N_A is the number of sources in area A, and A_k is the source k inside this area.

In this work the instantaneous phases of the signals were obtained using the Hilbert analytical signal, with 2000 samples of data at each side as padding in order to avoid edge effects. Mean and standard deviation FC matrices for each group are shown in Figure 4-1.

We checked the significance of each individual link for each group separately to ensure results interpretability using the analytical method depicted in (García-Prieto, Bajo, & Pereda, 2017; Wilkie, 1983). All the links were significant after FDR correction (Q=0.05) in the three diagnostic groups (all p-values < 0.028 for HC, all p-values < 0.033 for SCD and all p-values < 0.033 for MCI). Furthermore, in order to evaluate whether between-groups FC differences could be explained by source leakage, the Pearson correlations between beamformer weights were computed for each link and compared across groups (Garcés et al., 2014).

4.4.8. Network calculation

We calculated the FC in two key RSN: DAN and DMN. The coordinates of the nodes forming each RSN were defined in MNI space according to previous work (Jimenez et al., 2016; Watanabe et al., 2013). In turn, ROIs belonging to the DMN were subdivided into an anterior component (aDMN) and a posterior component (pDMN) (Andrews-Hanna et al., 2007). The representative FC for each RSN was computed by averaging the FC values over all pairs of regions comprising the corresponding network.

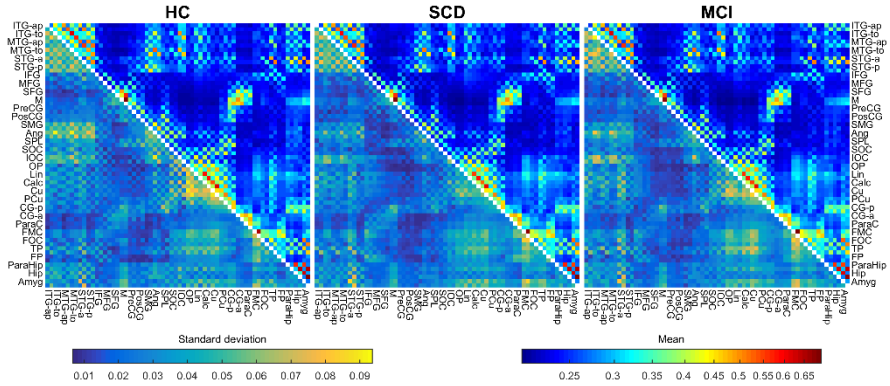

Figure 4-1. Mean and standard deviations of the connectivity values for HC (left), SCD (middle) and MCI (right) groups. The upper triangular area of each matrix shows the mean connectivity value for each link, using a logarithmic scale. The lower triangular area of each matrix shows the standard deviation for each link using a linear scale. Metrics use a different color map to avoid confusion.

4.4.9. Statistical analysis

In order to assess the differences in connectivity between groups we used an ANCOVA analysis with age as covariate. To ensure the accuracy of the results the ANCOVA analysis was non-parameterized using permutations. The original F value obtained with the ANCOVA analysis and the original grouping were stored, and a series of 10,000 randomizations of subjects were performed, preserving the original group sizes. For each randomization a new F value was obtained, which allowed creating a null-distribution of F values adapted to the characteristics of the data. This distribution was used to calculate the permutation-corrected *p*-value associated with the original F value.

Due to the high number of comparisons, of the order of 2,000, the probability of obtaining an elevated number of false positives is high. In order to avoid this problem we complemented the ANCOVA analysis with a False Discovery Rate (FDR) (Benjamini & Hochberg, 1995) multiple comparisons correction, using a *Q* value of 0.05 (a 5% rate of false positives).

Finally, for those links who survived the FDR correction we performed a pairwise comparison analysis with Tukey's Honestly Significant Difference correction to determine the groups in which the connectivity values were significantly different. These results were permutation-corrected in a similar way that the ANCOVA results.

Average FC values for each RSN were compared across groups employing a similar procedure with ANCOVA analysis non-parametrized using permutations.

We performed an ANOVA statistical test with FDR, using a *Q* value of 0.05, to identify and discard those links whose connectivity value is below the random threshold. Regarding the beamformer weights correlation, we employed a similar procedure to identify links whose correlation between beamformer filters differed among groups.

Last, we conducted correlation analysis in our sample using Pearson coefficient to characterize the relationship between the observed FC changes and cognitive state, as well as hippocampal volume. In order to address the multiple comparisons problem, all *p*-values were also corrected using FDR.

4.5. Results

4.5.1. Connectivity values

The FC analysis in this work was performed between anatomical areas, using a reduced version of the Harvard-Oxford atlas, consisting on the regions and abbreviations shown in Table 4-2 (*appended at the end of this chapter because of table size*). All the links were included in the analysis, as none of them was discarded because of a non-significant PLV value. The ANCOVA analysis brought significant between-group differences for 17 links in the overall comparison, FDR (Q=0.05) corrected (Figure 4-2). When observed in the pairwise comparison, the results showed a posterior network, with decreased connectivity, and an anterior network, with increased connectivity for both SCD and MCI compared to the HC group.

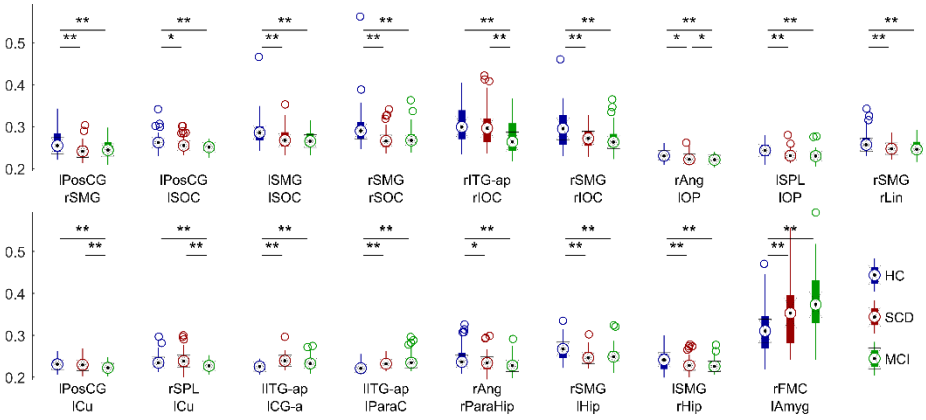

Figure 4-2. Distribution of connectivity values in each significantly different link for HC (blue), SCD (red) and MCI (green) groups. Asterisks mark significantly different

connectivity values between groups. One asterisk (*) denotes a *p*-value between 0.05 and 0.01. Two asterisks (**) denote a *p*-value lower than 0.01.

HC vs. MCI

In this comparison between HC subjects and MCI patients, all 17 links showed significant group effect in the pairwise comparison ($p<0.05$) (Figure 4-3). The anterior network presented higher FC in the MCI group compared to the HC group in three links connecting anterior regions, including left Inferior Temporal Gyrus, left Paracingulate and left Anterior Cingulate.. The posterior network exhibited lower FC in the MCI group, and comprised 14 links between connecting posterior cortical structures such as: temporal medial structures (both hippocampi and right parahippocampus), parietal (left Postcentral Gyrus, both Supramarginal Gyri) and occipital areas (left Frontal Pole, both Superior Occipital cortices, right Inferior Occipital Cortex, right Lingual Cortex).

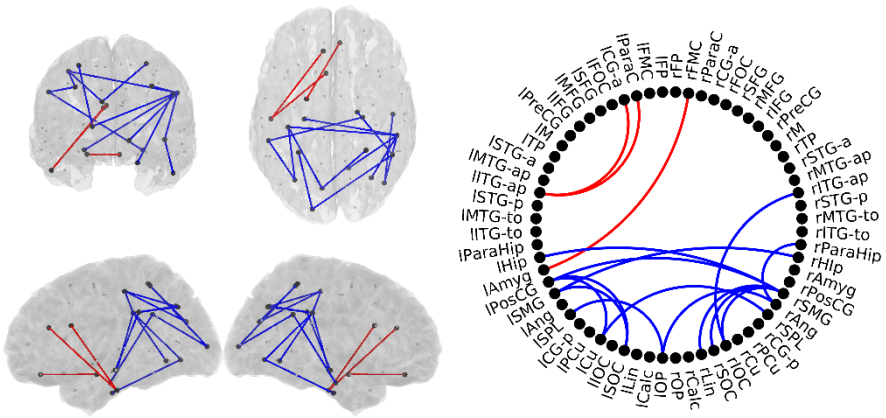

Figure 4-3. Links with significantly different FC values comparing Healthy Controls (HC) to Mild Cognitive Impairment (MCI) group. Left: Posterior, superior, left and right views of the brain. Right: Circle plot shows a schematic view of the significant links. Red lines indicate an increased FC value of MCI in respect to HC. Blue lines indicate a decreased FC value of MCI in respect to HC.

HC vs. SCD

In this comparison we identified a similar pattern with two sub-networks, as shown in Figure 4-4. SCD subjects showed increased FC values respect to the HC group ($p<0.05$) in three links connecting the same regions described in the previous comparison. SCD subjects also showed decreased FC in 11 links ($p<0.05$). Those links connected both intra- and inter-hemispherical areas between posterior regions. Interestingly, all the links affected in the SCD group were also disrupted in the MCI group in a similar manner.

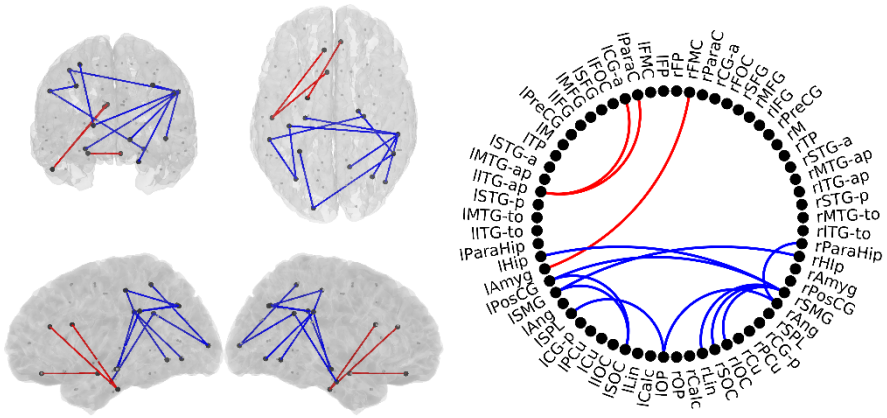

Figure 4-4. Links with significantly different FC values comparing Healthy Controls (HC) to Subjective Cognitive Decline (SCD) group. Left: Posterior, superior, left and right views of the brain. Right: Circle plot shows a schematic view of the significant links. Red lines indicate an increased FC value in SCD respect to HC. Blue lines indicate a decreased FC value of SCD in respect to HC.

MCI patients showed a network comprising four links where FC values were significantly lower ($p<0.05$) compared to SCD FC values (Figure 4-5). This network with reduced FC connected temporal, parietal and occipital regions of the brain, and comprised both intra- and inter-hemispheric links.

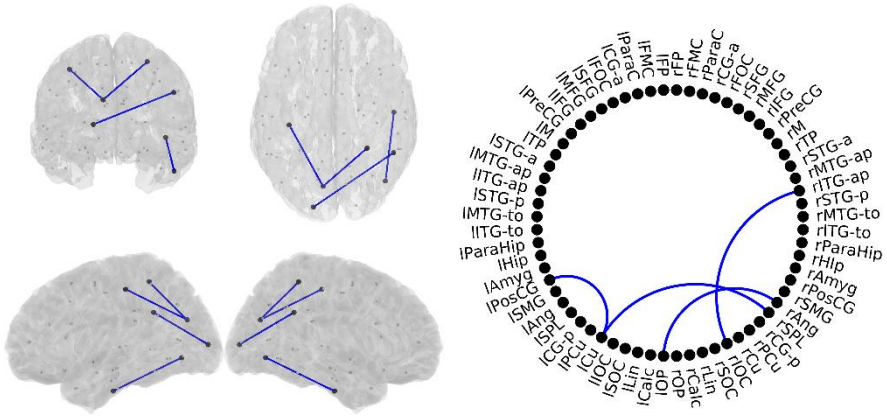

Figure 4-5. Links with significantly different FC values comparing Healthy Controls (HC) to Subjective Cognitive Decline (SCD) group. Left: Posterior, superior, left and right views of the brain. Right: Circle plot shows a schematic view of the significant links. Red lines indicate an increased FC value in SCD respect to HC. Blue lines indicate a decreased FC value of SCD in respect to HC.

4.5.2. Differences in RSN

Regarding DAN we observed a significant group effect ($p<0.05$). Pairwise comparisons revealed a FC decrease in DAN with respect to HC group in both SCD ($p<0.05$) and MCI ($p<0.01$). Mean FC of the DAN was not significantly different between SCD and MCI (Figure 4-6).

DMN was divided into two sub-networks and compared separately. We did not observe any significant group effect for the aDMN component. On the contrary, we found a significant group effect in pDMN ($p<0.005$). FC was significantly decreased in both SCD ($p<0.005$) and MCI ($p<0.005$) with respect to control. SCD and MCI did not show significant differences in mean FC in this sub-network (Figure 6).

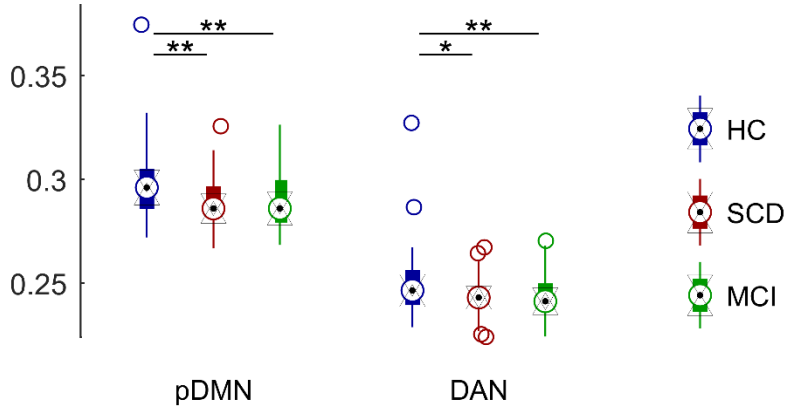

Figure 4-6. Distribution of mean connectivity values for each significantly different resting state network for HC (blue), SCD (red) and MCI (green). Asterisks mark significantly different connectivity values between groups. One asterisk (*) marks a *p*-value between 0.05 and 0.01. Two asterisks (**) mark a *p*-value lower than 0.01.

4.5.3. Correlation of beamformer weights

The correlation analysis between the beamformer weights determined there were no differences in the beamformer filters weights of the three groups. Taking this result into account, it is unlikely that the difference in the connectivity metrics could be caused by source leakage (Garcés et al., 2014).

4.5.4. Differences in hippocampal volume

Due to its extended use in the clinical diagnosis of MCI and AD, we completed this work by analyzing the hippocampal volumes of the participants. The corrected hippocampal volumes were compared between groups by means of an ANCOVA using the age as covariate. The contrast revealed significant differences between groups ($p<0.01$) (Table 4-1).

Pairwise comparisons showed that MCI patients had significantly lower volumes than both SCD ($p<0.01$) and HC group ($p<0.01$), while the volumes of SCD and HC participants did not differ ($p>0.05$).

4.5.5. Correlation analyses

Some of the links with a reduction in FC in SCD and MCI exhibited positive associations with hippocampal volume, MMSE, inverse digits test and BNT. This means that those subjects with lower FC values demonstrated an overall worse cognitive status, performed worse in working memory, executive functioning and language, and had smaller hippocampi. On the other hand, all three links with increased FC in SCD and MCI showed negative associations with BNT performance, meaning that those subject with larger FC hyper-synchronization in these specific links performed worse in language and executive functioning. All significant correlations and links are shown in Table 4-3 (*appended at the end of this chapter because of table size*)..

4.6. Discussion

In the present work, we have showed that elders presenting with SCD, in spite of their normal neuropsychological performance, exhibit a pattern of FC alterations similar to that shown by MCI patients. In addition, FC alterations were not restricted to DMN regions; they also affected broader areas largely unexplored in previous literature. When compared to the HC group, MCI patients exhibited a marked decrease in FC in the alpha band over posterior areas accompanied by an increased FC in anterior-ventral regions of the brain. The spatial distribution of alterations exhibited by the SCD group mimicked MCI patients' network disruption. According to our results, disconnection among posterior brain regions is even more pronounced in MCI patients when compared to SCD elders. These results draw a continuum in the preclinical phases of the disease where connectivity disruptions would take origin in the very early phases of AD (SCD stage), characterized by initial anterior FC increases and posterior FC decreases, followed in later stages by further posterior decreases in FC. Additionally, we obtained significant correlations of these FC changes with cognition and hippocampal volume, highlighting their pathological nature. To the best of our knowledge, this is the first time that whole brain FC impairment in the alpha band is reported in SCD patients. These results suggest that a subjective feeling of cognitive worsening with a preserved cognitive function could represent an early indicator of preclinical AD pathology in the brain.

It should be noted that most of the existing literature addressing FC in AD has focused specifically on DMN, and more work is needed to characterize

whether those findings are specific to DMN areas (Zarahn, Rakitin, Abela, Flynn, & Stern, 2007). We found decreased FC in SCD and MCI involving several areas classically related to the DMN, such as angular gyrus, medial temporal structures (i.e. hippocampus and parahippocampal cortex) or lateral inferior parietal areas. These connectivity decreases over DMN critical areas have been extensively reported (Hsiao, Wang, Yan, Chen, & Lin, 2013; Sorg et al., 2007; Zarahn et al., 2007). In addition, MCI patients showed decreased FC between parietal regions and occipital regions consistent with recent findings reporting decreased FC between DMN regions and visual network (Cai et al., 2016). Beyond marked decreases among posterior cortical regions, MCI patients also showed increased FC over anterior-ventral areas, involving temporal and frontal structures. Initial reports demonstrated that aging was associated with posterior decrements in FC and unspecific anterior increases and decreases (D. T. Jones et al., 2011). In this same study, AD patients exhibited a greater FC decrease over posterior regions and only increases among anterior brain regions, which is in line with our findings in the MCI and SCD groups. This anteroposterior dual pattern of hyper- and hypo-connectivity, respectively, has been described as a common feature of the network failure starting in pre-dementia stages and progressing along the AD continuum (Jones et al., 2015).

The most novel and relevant finding of our study is the disruption in brain FC in SCD participants. This pattern of alterations seems to be consistent with findings in AD literature. Our analysis revealed a posterior disconnection over lateral inferior parietal, medial temporal and occipital areas in SCD elders compared to the HC group. Furthermore, SCD participants exhibited an anterior hyper-synchronization affecting the exact same regions where MCI patients showed hyper-synchronization. To date, there are very limited yet contradictory results about the evolution of FC in SCD (Hafkemeijer et al., 2013; Wang et al., 2013), and no study has addressed resting state FC with MEG or EEG. However, our results seem to support previous literature in at-risk of AD populations describing similar anterior-ventral elevated FC and posterior diminished FC in prodromal stages of AD (Brier et al., 2012) and in cognitively normal APOE ε4 carriers (Machulda et al., 2011).

Interestingly, when comparing SCD and MCI groups, we found decreased FC in MCI subjects between occipital, inferior temporal and parietal regions. These results highlight the progressive FC loss throughout AD different stages, consistent with the conception of AD as a disconnection syndrome occurring along a continuum (Delbeuck et al., 2003). Furthermore, the localization of this progressive disconnection is consistent with previous work proving, with a very large sample, that posterior DMN subsystem connectivity declines linearly throughout the course of the

disease (D. Jones et al., 2015). Additionally, our results add value extending these findings to other brain regions not limited to DMN, and more importantly, to the very early preclinical stages of the disease in the SCD and MCI stage. It is worth noting that the anterior hyper-synchronization observed in SCD and MCI groups did neither progress nor decrease from SCD to the theoretically subsequent MCI stage. This is again in line with the above-mentioned work and others (Damoiseaux, Prater, Miller, & Greicius, 2012). Anterior hyper-synchronization across the entire AD spectrum appeared to have a trend towards declining in later stages of the disease. Our results seem to suggest that this increase in FC observed in early AD could actually take place in the preclinical stage of the disease, before clinical symptoms onset, to then slope towards MCI and early AD stages before the subsequent decline in FC already described in late AD.

The clinical impact of the AD-related anterior hyper-synchronization has been highlighted over the past few years. Traditionally, these alterations have been interpreted as a compensatory mechanism (Mormino et al., 2011). Alternatively, it has been proposed that cortical hubs, while acting as critical nodes of the network may augment the pathological cascade in AD increasing the deposition of Aβ (Buckner et al., 2009). In the same vein, these hyper-connected regions could represent the fingerprint of noisy or inefficient synaptic transmission. This synaptic burden could be propagated to downstream networks leading to what has been termed as a cascading network failure in AD (D. Jones et al., 2015). In fact, higher levels of Aβ accumulation can be caused by increases in synaptic activity (Cirrito et al., 2008). Furthermore, previous MEG results showed that hyper-synchronization of anterior cingulate with certain brain regions predicted a fast conversion from MCI to AD (López et al., 2014). In our work, SCD and MCI anterior alterations involved areas classically related to the salience network. Interestingly, FC increases in these brain regions have been linked with mood disorders in mild AD patients (Balthazar et al., 2014). The association of SCD and certain psychiatric conditions has represented a controversial topic in the field (Buckley et al., 2013). However, cognitive concerns along with these affective symptoms could represent a common susceptibility towards AD as has been already suggested (Snitz et al., 2015). The pathological nature of this hyper-synchronization is reinforced by our correlation results revealing that those subjects with higher synchronization values in these links performed worse in an executive function and language task.

After exploring whole brain FC we focused on two RSN´s crucial to AD progression and understanding. Attentional networks (ventral and dorsal) are known to be impaired in AD (Li et al., 2012). However, we studied DAN as it was the only one consistently disturbed in MCI patients in previous literature (Qian, Zhang, Li, & Sun, 2015; Zhang et al., 2015). Our

analysis revealed a significant decrease in the mean FC of DAN in MCI patients supporting previous results. However, our results suggest that this decrease seems to occur in an early stage, when elders still present only cognitive concerns, and maintain this level of hypo-synchronization at least until MCI stage. Alterations in DAN has been related to deficits in top-down attentional control in AD (Zhang et al., 2015).

DMN is considered the key RSN in AD progression. In fact, Aβ spread has been topologically linked to this network (Grothe, Teipel, & Alzheimer's Disease Neuroimaging Initiative, 2016). The role of posterior cingulate cortex (PCC) in AD progression has received major attention, as it is a crucial hub of pDMN, and it is often used as a seed for DMN studies. The vast majority of previous studies agree that PCC is progressively disconnected from other brain regions inside DMN, reporting FC decreases in AD and MCI patients with either fMRI (Kim et al., 2016; Toussaint et al., 2014; Weiler et al., 2014), a combination of DTI and a functional technique (Garcés et al., 2014; Soldner et al., 2012) or with EEG (Hsiao et al., 2013). However, literature is not without some controversy, as some studies reported an increase in directional connectivity from PCC to other posterior brain regions in MCI. Our results support the majority of studies reporting a decrease in FC over pDMN in the AD-continuum. According to our results, FC alterations start in SCD phase, and remain constant along MCI stage. Of note, we did not observe any significant change in aDMN, which is in line with some previous work (Kim et al., 2016; Song et al., 2013), but not all (D. Jones et al., 2015). This discrepancy could be explained by the pathological stage of the sample studied, which usually consists of AD patients, thus in a more severe phase of the disease than our sample. Additionally, it has been mentioned that the antero-posterior FC dual change pattern could not be limited to DMN regions (Wiepert et al., 2017), which looks to be confirmed by our results.

FC deficits have been previously linked to structural abnormalities in MCI patients (Garcés et al., 2014). Although we did not address structural connectivity, we observed a significant decrease in hippocampal volume in our MCI group. Noteworthy, hippocampal volume correlated positively with FC in two hypo-synchronized links, in a way that those subjects with smaller hippocampi showed more aberrant FC values. No changes in hippocampal volume were observed in SCD elders, underscoring the ability of MEG FC to detect subtle brain changes before structural alterations become evident.

This work constitutes the first report of whole-brain FC alterations in SCD with a high temporal resolution technique such as MEG. These FC disruptions affected AD typically-related areas and showed great overlap with MCI alteration pattern, but with a milder intensity. Furthermore, DAN

and pDMN were affected in both SCD and MCI, showing a similar level of FC decrease. This is in agreement with previous neuroimaging findings reporting a continuum in the preclinical stages of AD, namely a relatively progressive increase in the burden of certain biomarkers increasing from healthy aging, to SCD and then MCI such as reduced metabolism (Scheef et al., 2012) or brain atrophy (Saykin et al., 2006). Future work should address the predictive value of these alterations, and test whether FC disruption represents a hallmark of AD pathology, increasing the likelihood of MCI and SCD elders to develop further stages of the AD.

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4.7. Experiment 2 appendix

Abbreviation	Full name
Amyg	Amygdala
Ang	Angular Gyrus
Calc	Calcarine cortex
CG	Cingulate Gyrus
Cu	Cuneal Cortex
FMC	Frontal Medial Cortex
FOC	Frontal Orbital Cortex
FP	Frontal Pole
Hip	Hippocampus
IOC	Inferior Lateral Occipital Cortex
ITG	Inferior Temporal Gyrus
ITG	Inferior Frontal Gyrus
Lin	Lingual Gyrus
M	Motor cortex
MFG	Middle Frontal Gyrus
MTG	Middle Temporal Gyrus
OP	Occipital Pole
ParaC	Paracingulate Gyrus
ParaHip	Parahippocampal Gyrus
PCu	Precuneous
PosCG	Postcentral Gyrus
PreCG	Precentral Gyrus
SFG	Superior Frontal Gyrus
SMG	Supramarginal Gyrus
SOC	Superior Lateral Occipital Cortex
SPL	Superior Parietal Lobule
STG	Superior Temporal Gyrus
TP	Temporal Pole

Table 4-2. **List of ROIs of the anatomical atlas.** Correspondence between abbreviations showed in Figure 4-1, Figure 4-2 and Figure 4-3 and regions depicted in the Harvard-Oxford anatomical atlas (Desikan et al., 2006). Preceding letter l or r stands for left or right hemisphere, respectively. Abbreviations ending in -a, -p, -ap or -to stand for anterior part, posterior part, antero-posterior part or temporo-occipital part, respectively.

	Hippocampal volume	MMSE	Inverse digits	BNT - Spontaneous responses
lPosCG lSOC	r = 0.2817 *p* = 0.0012	*n.s.*	*n.s.*	*n.s.*
lSMG lSOC	r = 0.2504 *p* = 0.0041	r = 0.2262 *p* = 0.0094	n.s.	r = 0.2350 *p* = 0.0076
rSMG rSOC	*n.s.*	r = 0.2567 *p* = 0.0031	*n.s.*	*n.s.*
rITG-ap rIOC	*n.s.*	*n.s.*	*n.s.*	r = 0.2783 *p* = 0.0015
rAng lOP	*n.s.*	*n.s.*	r = 0.2411 *p* = 0.0059	*n.s.*
lSPL lOP	*n.s.*	*n.s.*	r = 0.3003 *p* = 0.0005	*n.s.*
rSMG lHip	*n.s.*	*n.s.*	*n.s.*	r = 0.2651 *p* = 0.0025
lSMG rHIp	*n.s.*	*n.s.*	*n.s.*	r = 0.2343 *p* = 0.0078
lITG-ap lCG-a	*n.s.*	*n.s.*	*n.s.*	r = -0.3118 *p* = 0.0003
lITG-ap lParaC	*n.s.*	*n.s.*	*n.s.*	r = -0.3615 *p* = 0.0000
rFMC lAmyg	*n.s.*	*n.s.*	*n.s*	r = -0.2602 *p* = 0.0030

Table 4-3. **Results of the correlation analysis between connectivity values and neuropsychological scores.** Correlation values of each significant correlation between the significantly different links and the anatomical values and neuropsychological scores. First 14 rows depict hypo-synchronization links. Last 3 rows depict hyper-synchronization links. The signification values were FDR corrected (Q=0.05) and the significance threshold was subsequently placed at $p = 0.0094$. *n.s.* indicates non-significant correlations. MMSE stands for Mini Mental State Examination. BNT stands for Boston Naming Test.

4.8. Summary of conclusions

1. The results showed a marked disruption in brain connectivity in the SCD group. These alterations included both hypo-synchronization and hyper-synchronization between different brain regions.

2. We were able to replicate previous findings in AD literature reporting FC alterations in MCI patients. We also observed areas showing increased FC while the communication between other brain regions was dampened as compared to HC elders.

3. The observed pattern of disruption in SCD clearly paralleled that obtained for MCI patients. When we compared each of those groups to HC we identified two clearly isolated subnetworks showing different alterations. On the one hand a large posterior network emerged in both comparisons characterized by a significantly reduced FC in our suspected prodromal AD groups. This network included several occipital, parietal and temporal regions showing decreased FC in both SCD and MCI compared to HC. Further, an anterior network comprising anterior cingulate and inferior temporal regions among others showed increased FC in both SCD and MCI compared to HC. However, what is most important about these results is the fact that the alterations found in SCD were remarkably similar to those observed in MCI, which clearly highlights that SCD should share some pathological characteristics with MCI, and thus AD.

4. SCD stage could represent the initial stages of the proposed cascading network failure along AD continuum. Previous literature supports that cognitive concerns could potentially represent a marker for AD but whether SCD already showed signs of network failure remained ignored. Our results support that the hypersynchronization known to occur in the early AD stages is already present in the SCD stage and does not seem to increase towards MCI. Furthermore, the initial decrease in FC values over posterior regions existent in SCD is further diminished in MCI patients, which is again consistent with the disconnection hypothesis of AD.

5. DMN and DAN are both affected in the preclinical stages of the disease, namely in SCD elders. Furthermore, even though MCI patients showed significant decreases in the connectivity in these two networks, their network integrity was not further compromised compared to SCD. This again points towards an early appearance of FC disruption throughout the course of the disease.

6. Posterior DMN connectivity is impaired in SCD and MCI groups. We did not observe alterations in the aDMN. This highlights that, first, DMN components are selectively affected in AD, and second, that anterior FC disruptions might not be limited to DMN regions as predominantly studied in fMRI literature.

7. There is a clear and significant relationship between brain pathological FC patterns and cognitive deterioration in AD. More concretely, it is worth highlighting that anterior hyper-synchronization was selectively related to language impairment. Furthermore, posterior desynchronization is associated with an overall worse cognitive state, memory performance and language.

8. Lower hippocampal volume was associated with bigger decreases in FC over posterior regions. This result highlights the pathological essence of these FC alterations. Noteworthy, this associations are highly consistent with the fact that posterior desynchronization, as opposed to anterior hyper-connection, progresses from SCD to MCI, temporarily overlapping with the gray matter impairment onset.

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5. Experiment 3

Network analysis in SCD and MCI

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5.1. Summary of objectives

1. Brain networks are known to be affected in AD patients however very little is known about network functioning in the preclinical stages. The main objective of this study was to evaluate if network alterations are already present in the SCD stage and how they eventually progress towards later stages when patients become MCI. This general objective is further developed in the following subsequent specific objectives:

    a. Brain networks are known to show a highly efficient and specific structure in which a high number of short range connections combined with a sufficient number of long range links ensure an optimal communication among brain regions. This small-world structure, as it is referred in the literature, is known to be affected in AD, and also in MCI, but has never been studied in SCD elders. We intended to replicate previous results in MCI patients, to add spatial resolution by using MEG source space analysis, and to evaluate the small-world brain structure in SCD elders for the first time.

    b. The integrity of short range connections and closed connectivity loops between neighboring brain regions can be measured using clustering or transitivity metrics. One of the objectives of this work is to evaluate if this network property is already affected in SCD patients. Furthermore, we wanted to confirm changes in the expected direction in MCI, which has been reported to show decreased local connectedness. Lastly, it is important to integrate the information of the changes observed in both groups to draw the line of the possible evolution of this network property along AD preclinical stages.

    c. This study seeks to determine if the modular and hierarchical architecture, which is known to govern normal brain functioning, is altered in SCD and/or MCI. We compared the extent to which each of the different group´s brain organization followed this modular structure.

2. Global network metrics allow the characterization of general brain patterns and the description of the whole network with a single parameter. However, this study also aims to elucidate the concrete spatial pattern of network alterations by evaluating these properties at the node level. More concretely:

a. We wanted to evaluate if SCD and MCI showed any sign of network strength readjustment by measuring the degree centrality of each of their nodes

b. This study evaluated the pattern of local connectedness at each brain region. We aimed to investigate whether local connectedness changes occur in the SCD and/or MCI stage and the specific localization of these hypothetical alterations.

c. In this study the specific allocation of each brain region to one brain module was estimated and compared across groups. By assessing this network property we wanted to evaluate how brain networks are reorganized in the context of initial AD pathology and if this hypothetical reorganization served a compensatory or pathological process.

3. The third major objective of experiment III was to evaluate the possible functional meaning of the changes observed in network structure. It is known that normal brain functioning, and cognitive processes are supported by these brain properties. As a consequence, we explored the implications of the observed network functioning for SCD and MCI cognitive functioning.

5.2. Abstract

Introduction: Subjective Cognitive Decline (SCD) is a largely unknown state thought to represent a preclinical stage of Alzheimer´s Disease (AD) previous to mild cognitive impairment (MCI). However, the course of network disruption in these stages is scarcely characterized.

Methods: We employed resting state magnetoencephalography in the source space to calculate network smallworldness, clustering, modularity and transitivity. Nodal measures (clustering and node degree) as well as modular partitions were compared between groups.

Results: The MCI group exhibited decreased smallworldness, clustering and transitivity and increased modularity in theta and beta bands. SCD showed similar but smaller changes in clustering and transitivity, while exhibiting alterations in the alpha band in opposite direction to those showed by MCI for modularity and transitivity. At the node level MCI disrupted both clustering and nodal degree while SCD showed minor changes in the latter. Additionally, we observed an increase in modular partition variability in both SCD and MCI in theta and beta bands.

Discussion: SCD elders exhibit a significant network disruption, showing intermediate values between HC and MCI groups in multiple parameters. These results highlight the relevance of cognitive concerns in the clinical setting and suggest that network disorganization in AD could start in the preclinical stages, before the onset of cognitive symptoms.

5.3. Introduction

Alzheimer´s Disease (AD) is the most common cause of dementia (Blennow, de Leon, & Zetterberg, 2006). It is an insidious and neurodegenerative disease that produces a progressive decline in multiple cognitive domains, specially episodic memory (Peña-Casanova, Sánchez-Benavides, de Sola, Manero-Borrás, & Casals-Coll, 2012). There is a growing interest in the preclinical stages of the disease due to the lack of a curative treatment (Iqbal, Liu, & Gong, 2014) and the dramatic increase expected in the number of cases in the future (Ferri et al., 2005).

AD is considered a disconnection syndrome (Delbeuck, Linden, & Collette, 2003). Accordingly, many studies have revealed a progressive loss of functional connectivity (FC) between key brain regions in AD patients (Damoiseaux, Prater, Miller, & Greicius, 2012; D. Jones et al., 2015). For this reason, a network perspective is suitable to characterize the specific patterns of alterations affecting the connections between different brain regions in the AD continuum. Given the large amount of data derived from this kind of analyses, graph theory has become a very powerful tool in neuroimaging to summarize and study the organization of whole brain networks and capture the alterations in the network structure caused by the disease (Filippi et al., 2013). Graph theory describes a network as a set of nodes, the components of a system (represented in the brain network perspective as a region of interest-ROI), and a number of edges, representing the connection between each pair of nodes (Rubinov & Sporns, 2010; Sporns, Chialvo, Kaiser, & Hilgetag, 2004).

The brain exhibits a particular organization characterized by a large number of short range connections between related areas while maintaining some long range connections between less related areas. This *small-world* topology (Watts & Strogatz, 1999) is especially suited for cognitive processing (Danielle S. Bassett & Bullmore, 2016) and is the expression of a trade-off between the pressure to minimize wiring cost (i.e. reducing long range axons) and maximizing information flow and integration (Bullmore & Sporns, 2012). Alzheimer´s causes major disruption in network organization, affecting not only this small-world architecture of the brain (Sanz-Arigita et al., 2010a; Stam et al., 2008), but also other brain network properties like clustering or modularity (Matthew R. Brier et al., 2014).

Graph theory can help us unravel the degenerative process occurring during the extended preclinical phase of AD, which could start over 15 years before the onset of clinical symptoms (Jack et al., 2013). Mild cognitive impairment (MCI) is a well-known at-risk stage of AD, characterized by a slight although detectable cognitive decline in one or more cognitive domains (Ronald C Petersen, 2016). MCI patients exhibit multiple

evidences of AD pathology such as cortical atrophy (Ma et al., 2016), β-amyloid depositions (Edmonds et al., 2016) or connectivity disruption (Maestú et al., 2015). This brain pathology translates into network alterations similar to those exhibited by AD patients (Pereira et al., 2016; Tijms et al., 2013).

Earlier in the course of the disease, healthy elders with normal neuropsychological performance could manifest a subjective feeling of cognitive worsening. The inclusion of this stage, coined as subjective cognitive decline (SCD) (Jessen et al., 2014), in the preclinical asymptomatic stage of AD still remains a matter of debate (Röhr, Luck, Villringer, Angermeyer, & Riedel-Heller, 2017). Although some studies did not find evidences of AD pathology in the brain of SCD elders (R Buckley et al., 2013; Rowe et al., 2010), there is a growing body of literature supporting the link between cognitive concerns and characteristic AD signs such as β-amyloid accumulation (Mielke et al., 2012), reduction of glucose metabolism in AD related areas (Scheef et al., 2012) and relative power decreases in alpha band (D. López-Sanz et al., 2016) among others. Furthermore, a meta-analysis combining data from more than 29000 subjects has revealed that healthy elders with SCD are twice as likely to develop dementia than individuals without cognitive concerns (Mitchell, Beaumont, Ferguson, Yadegarfar, & Stubbs, 2014). The conversion rate to AD for SCD is even greater when segregating healthy elders with positive β-amyloid into SCD and non SCD (Rachel Buckley et al., 2016). However, very little is known about the beginning of network disruption in this stage. The only study to date addressing this issue employed structural networks to build graph matrices, reporting no differences between healthy elders with and without cognitive concerns (X.-N. N. Wang et al., 2016). Although from a theoretical perspective graph metrics provide a common framework that allows comparison between networks coming from different types of data (Tijms et al., 2013), evidence suggests that differences in methodology and relatively small sample sizes may produce divergent results (Matthew R. Brier et al., 2014).

To the best of our knowledge this is the first study to date employing graph metrics built from functional data to characterize the evolution of network dynamics throughout the preclinical stages of AD including healthy elders without cognitive concerns (HC), healthy elders with SCD, and MCI patients. To this aim, we employed resting state activity recorded with magnetoencephalography (MEG), and reconstructed source level activity to precisely detect and localize changes in brain network properties and modular organization. Electrophysiological measures of synchrony, derived from EEG or MEG have been proven useful in the detection of different pathologies (Chyzhyk, Graña, Öngür, & Shinn, 2015; Correas et al., 2015; Coullaut-Vallera et al., 2014), and specifically in AD (Ahmadlou,

Adeli, & Adeli, 2010; Houmani, Dreyfus, & Vialatte, 2015). Based on previous imaging studies, we expected to detect altered network properties in MCI patients with respect to HC. More importantly, we hypothesized that SCD elders will show alterations in the same direction of those exhibited by MCI patients, although to a lower extent, thus exhibiting intermediate values between HC and MCI.

5.4. Methods

5.4.1. Subjects

The sample for this study was recruited from two recent projects funded by the Spanish Ministry of Economy and Competitiveness. In total, 187 elders were included in this study recruited from three centers: the neurology department at "Hospital Clínico San Carlos", the "Center for cognitive impairment prevention" and the "Seniors center of Chamartín District", all of them in Madrid, Spain. Participants were divided into three groups: 63 healthy elders with neuropsychological performance within the normal range and no subjective feeling of cognitive decline (HC), 55 healthy elders with unimpaired cognitive performance but with SCD and 69 elders with Mild Cognitive Impairment (MCI). Subjects were aged between 60 and 81 years old. Table 5-1 summarizes relevant clinical and demographic data. The exclusion criteria to enroll in the study included the following: 1) history of psychiatric or neurological disorders or drug consumption that could affect MEG activity such as cholinesterase inhibitors, 2) evidence of infection, infarction or focal lesions in a T2-weighted scan within 2 months before MEG acquisition 3) a modified Hachinski score equal to 5 or higher, 4) a GDS-SF score equal to 5 or higher, 5) alcoholism, chronic use of anxiolytics, neuroleptics, narcotics, anticonvulsants or sedative hypnotics. Furthermore, we conducted additional analysis to rule out other possible causes of cognitive decline such as B12 vitamin deficit, diabetes mellitus, thyroid problems, syphilis, or Human Immunodeficiency Virus (HIV). All participants signed an informed consent. This study was approved by the *Clínico San Carlos Hospital* ethics committee and the procedure was performed in accordance with approved guidelines and regulations.

5.4.2. Clinical assessment

An initial screening was carried out to assess the general functioning and cognitive status of the sample. This screening included The Mini Mental State Examination (MMSE) (Lobo, Ezquerra, Gómez Burgada, Sala, & Seva Díaz, 1979), the Hachinski Ischemic Score (HIS) (Rosen, Terry, Fuld, Katzman, & Peck, 1980), the Functional Assessment Questionnaire (FAQ) (Pfeffer, Kurosaki, Harrah, Chance, & Filos, 1982) and the Geriatric Depression Scale – Short Form (GDS-SF) (Yesavage et al., 1982).

	Mean ± SD			*p*-values		
	Control	SCD	MCI	HC - SCD	HC - MCI	SCD - MCI
Age (years)	70.7±4.5	71±5	71.9±4.2	-	-	-
Gender (M/F)	24/39	13/42	22/47	-	-	-
GDS	1.4±1.84	1.8±1.7	3.8±3.2	n.s.	$4.5 \cdot 10^{-8}$	$6.3 \cdot 10^{-6}$
Education	4.19±1	3.95±1	3±1.15	n.s.	$1.4 \cdot 10^{-9}$	$2.7 \cdot 10^{-6}$
MMSE	28.9±1.2	29±1	26.8±2.4	n.s.	$9.7 \cdot 10^{-10}$	$9.6 \cdot 10^{-10}$
Dir. digits	8.5±1.9	8.7±2	7.3±2.2	n.s.	0.004	0.0003
Inv. digits	6.2±1.9	5.6±2	4.3±1.5	n.s.	$3.3 \cdot 10^{-9}$	0.0002
TMTb hits	23.2±2.6	22.4±3.2	19.4±6.1	n.s.	$3.5 \cdot 10^{-6}$	$5.2 \cdot 10^{-4}$
TMTb time (s)	108.7±60.8	133±62	227.6±102.6	n.s.	$9.5 \cdot 10^{-10}$	$1.2 \cdot 10^{-9}$
Imm. recall	41.7±11	34.8±11	18.3±7.4	0.0005	$9.5 \cdot 10^{-10}$	$9.5 \cdot 10^{-10}$
Del. recall	25.1±8	20.4±8.6	7.5±6.2	0.001	$9.5 \cdot 10^{-10}$	$9.5 \cdot 10^{-10}$
Hipp. volume	$0.005 \pm 5 \cdot 10^{-4}$	$0.005 \pm 6 \cdot 10^{-4}$	$0.004 \pm 8 \cdot 10^{-4}$	n.s.	$2 \cdot 10^{-6}$	$2 \cdot 10^{-5}$

Table 5-1. **Demographic data.** The left half of the table shows mean ± SD (standard deviation). The right half of the table shows the *p*-values resulting of the significant between groups ANOVA comparisons. If the factor is not significant a hyphen is shown. If a specific post-hoc comparison is not significant ($\alpha = 0.05$) 'n.s.' is shown. Education was measured from 1 (illiterate) to 5 (university studies). GDS stands for Geriatric Depression Scale - Short Form. MMSE stands for Mini Mental State Examination, Dir. digits and inv. digits stand for direct and inverse digit span test respectively. TMTb stands for Trail Making Test form b. Imm. Recall and del. recall stand for immediate and delayed recall of the WMS-III respectively. Hipp. Volume stands for normalized hippocampal volume.

Each of the participants underwent an extensive neuropsychological assessment, whereupon in the following weeks they completed a magnetic resonance imaging (MRI) scanner and a MEG scan. The neuropsychological assessment included: Direct and Inverse Digit Span Test (Wechsler Memory Scale, WMS-III), Immediate and Delayed Recall (WMS-III), Phonemic and Semantic Fluency (Controlled oral Word Association Test, COWAT), Ideomotor Praxis of Barcelona Test, Boston Naming Test (BNT) and Trail Making Test A and B (TMTa and TMTb)

and Rule Shift Cards (Behavioural Assessment of the Dysexecutive Syndrome, BADS).

MCI diagnosis was carried out according to the criteria established by Petersen (R C Petersen, 2004) and Grundman (Grundman, 2004). In addition, MCI subjects did not fulfill criteria for dementia diagnosis.

All the participants included into the HC or SCD group showed a normal cognitive performance in the neuropsychological tests. Information regarding cognitive concerns was collected during an interview with clinician experts, where subjects self-reported whether they felt a significant cognitive decline with respect to their previous performance level. The final group assignment was made by multidisciplinary consensus (neuropsychologists, psychiatrists and neurologists). Several possible confounders of SCD such as: medication, psycho-affective disorders or relevant medical conditions were taken into account for the decision. According to the SCD International Working Group (SCD-I-WG), all the participants were older than 60 at onset of SCD, which occurred within the last five years (Jessen et al., 2014).

5.4.3. MRI acquisition

A T1-weighted MRI was available for each subject, acquired in a General Electric 1.5 Tesla magnetic resonance scanner, using a high-resolution antenna and a homogenization PURE filter (Fast Spoiled Gradient Echo sequence, TR/TE/TI = 11.2/4.2/450 ms; flip angle 12°; 1 mm slice thickness, 256x256 matrix and FOV 25 cm). MRI images were processed with Freesurfer software (version 5.1.0) and its specialized tool for automated cortical and subcortical segmentation (Fischl et al., 2002) in order to obtain the volume of both hippocampi. The volume of both hippocampi was averaged and then normalized dividing it by the total intracranial volume to account for head volume differences between subjects.

5.4.4. MEG recordings and preprocessing

Electrophysiological data were acquired by using a 306 channel (102 magnetometers, 204 planar gradiometers) Vectorview MEG system (Elekta AB, Stockholm, Sweden), placed inside a magnetically shielded room (VacuumSchmelze GmbH, Hanau, Germany) at the "Laboratory of Cognitive and Computational Neuroscience" (Madrid, Spain). All recordings were obtained while subjects were sitting comfortably, resting awake with eyes closed. MEG acquisition consisted of four minutes of signal for each subject. During the recording continuous information of the head position, blinks and eye movements were acquired.

MEG data was registered using a sampling rate of 1000 Hz and an online anti-aliasing bandpass filter between 0.1 and 330 Hz. Recordings were processed offline using a spatiotemporal signal space separation algorithm with movement compensation (Taulu & Simola, 2006) (correlation window 0.9, time window 10 seconds) in order to remove magnetic noise originated outside the head.

We employed an automatic procedure from Fieldtrip package to detect ocular, muscular and jump artifacts (Oostenveld, Fries, Maris, & Schoffelen, 2011), and the artifact detection was visually confirmed by a MEG expert afterwards. The remaining data were segmented in 4 seconds epochs of artefact-free activity. Additionally, an ICA-based procedure was employed to remove the electrocardiographic component. Due to the redundancy between gradiometers and magnetometers after temporal-signal space separation (tSSS) filtering, only magnetometers were used for further analysis.

5.4.5. Source reconstruction

The source model consisted of 2459 sources placed in a homogeneous grid of 1cm in the Montreal Neurological Institute (MNI) template, then linearly transformed to subject space. The leadfield was calculated using a three-shell Boundary Element Method (brain-skull, skull-scalp and scalp-air interfaces generated from the subject's T1 MRI) computed with OpenMEEG software (Gramfort, Papadopoulo, Olivi, & Clerc, 2010).

MEG data were band-pass filtered in theta [4-8 Hz], alpha [8-12 Hz] and beta [12-30 Hz] bands using an 1800 order finite input response (FIR) filter designed using Hanning window. Our frequency band choice was based on previous studies reporting greater reliability of graph metrics in these specific bands (Deuker et al., 2009; Hardmeier et al., 2014; Jin, Seol, Kim, & Chung, 2011). Data was filtered in a two-pass procedure as implemented in Matlab´s filtfilt to avoid phase distortion. 2000 samples of real data at each side were employed as padding to avoid edge effects. Linearly Constrained Minimum Variance (LCMV) beamformer (Van Veen, van Drongelen, Yuchtman, & Suzuki, 1997) was employed to solve the inverse problem and calculate source time-series.

5.4.6. Functional connectivity calculation

First, source time series for 52 regions of interest (ROIs) were extracted using a data-driven functional atlas created by Craddock *et al.* (Craddock, James, Holtzheimer, Hu, & Mayberg, 2012). . The original atlas consisted of 60 ROIs extracted through spatially constrained spectral clustering, generating spatially coherent regions of homogeneous resting state FC. For our MEG analysis, only cortical ROIs were employed, resulting in 52 total

ROIs. The activity of each ROI was defined as the time series of the source within the ROI which showed the highest correlation with all its neighbours in the ROIs.

For each frequency band, the functional coupling between each pair of ROIs was estimated with envelope correlation with leakage correction (Brookes, Woolrich, & Barnes, 2012; Hipp, Hawellek, Corbetta, Siegel, & Engel, 2012). First, pairwise ROI time series were orthogonalized through a linear regression between both signals.. The orthogonalization solves the problem of the artifactual correlations generated by the ill-posed inverse problem, thus avoiding bias in FC estimates due to source leakage. Afterwards, the envelopes of the two orthogonalized time-series were calculated with the modulus of the Hilbert transform of the ROI time series, already band-pass filtered in the specific frequency band. The FC between any two ROIs was estimated as the Pearson correlation coefficient between their envelopes. Since the orthogonalization of the time series is non-symmetrical, the final FC estimate between two ROIs was defined as the average of the envelope correlation values obtained when using both ROI time series as seeds separately (Colclough, Brookes, Smith, & Woolrich, 2015).

5.4.7. Graph calculations

In general, a graph (G) is expressed as a set of nodes (V) and the connections between those nodes, or edges (E); G = (V,E). The connections between nodes are stored in a weight matrix (W). In this matrix, the weight (w) of the connection between the node *i* and the node *j* is captured by the element w_{ij} of the graph. When constructing weighted networks in neuroimaging, weak connections represent a major limitation in graph analysis because they may introduce spurious correlations into the matrix, adding noise into the network (Tijms et al., 2013). Consequently, we employed a binarized version of the original connection matrix of each subject using an arbitrary threshold (τ). Any connection with a value below the selected threshold was set to zero, those values higher than the threshold were set to one. $w_{ij} < \tau = 0$; $w_{ij} \geq \tau = 1$. AD and MCI patients are known to exhibit lower FC values (Mathew R Brier et al., 2012; Sankari, Adeli, & Adeli, 2012; J. Wang et al., 2013). As a consequence, their binarized matrices when employing a given FC threshold could be sparser than those of HC, which is known to significantly affect graph metrics (Sanz-Arigita et al., 2010a). In fact, MCI patients showed a decreased matrix density in the beta band (and both SCD and MCI showed a trend towards sparser networks in the theta band) when using a given FC value to threshold all the matrices. To avoid this effect we selected a critical value for each individual FC matrix such that the proportion of edges overpassing this threshold was set to a fixed percentage, or matrix density. Choosing a

specific threshold involves an arbitrary decision, therefore, according to Rubinov M. and Sporns O. (Rubinov & Sporns, 2010) it is considered good practice to explore changes in network topology across a wide range of matrix densities to ensure robustness of results. We set our threshold range from 5% to 35% of matrix edges to ensure quality and completeness of the data (D. S. Bassett & Bullmore, 2006). Similar ranges have been commonly employed in the literature according to Bassett D. and Bullmore E. (Danielle S. Bassett & Bullmore, 2016). To enable comparison between groups, we created a surrogate distribution of network parameters for each frequency band and subject, and normalized all value parameters by subtracting the mean surrogate value and dividing it by the surrogate standard deviation. Networks constructed using correlation show higher clustering values than random networks given the transitive nature of correlation. Thus it is important to use random networks that preserve these features characteristic of correlational networks. We employed the Hirschberger-Qi-Steuer (HQS) algorithm (Hirschberger, Qi, & Steuer, 2007), implemented for FC matrices in (Zalesky, Fornito, & Bullmore, 2012), which maintains the mean and variance of the off-diagonal elements and the mean of the diagonal elements of the FC matrix.

5.4.8. Network parameters

All network and nodal parameters were calculated using a freely available toolbox for Matlab: Brain Connectivity Toolbox (Rubinov & Sporns, 2010).

Small-world (S)

Brain networks are thought to reconcile a large amount of short range connections for segregation while maintaining a sufficient amount of long range connections to ensure processing integration. The small-world parameter of a binarized network is defined as the ratio between normalized clustering coefficient (C_{glob}) and normalized path length (L) with respect to a null model network (M D Humphries, Gurney, & Prescott, 2006). And thus represents the ratio between the amount of short range connectivity (or segregation) and the distance between any two nodes, i.e. network integration. The random networks for small-world calculation were generated using HQS algorithm, given the tendency of correlational networks to overestimate smallworldness if an inappropriate null model is employed.

$$S = \frac{C_{glob} / C_{rand}}{L / L_{rand}}$$

Global clustering coefficient (C_{glob})

Clustering coefficient is a measure of local connectivity representing the fraction of triangles surrounding a node. It was implemented in BCT as defined in (Watts & Strogatz, 1999). That is to say, the algorithm calculates how many of the nodes to which a node is connected, are also connected between them. C_{glob} is defined as the mean clustering of all nodes. The mean clustering coefficient is then normalized individually for each node, thus being possibly biased by the presence of nodes with a low degree. This is the clustering definition employed:

$$C_i = \frac{2E_i}{k_i(k_i - 1)}$$

where C_i is the clustering of each node which is averaged afterwards to obtain C_{glob}, E_i is the number of edges between the neighbors of i and k_i is the degree of node i, i.e. the number of neighbors the node has.

Transitivity (T)

Transitivity, similarly to clustering, reflects how well a node is connected to its neighboring areas. However, unlike clustering coefficient, transitivity is quite robust against the presence of barely connected nodes. Instead of being normalized individually for each node, transitivity is normalized by the value of the whole network (Newman, 2003), thus being more resistant to the presence of nodes with low degree. Transitivity was calculated following the definition in (Mark D. Humphries & Gurney, 2008);

$$T = \frac{3\ x\ number\ of\ triangles}{number\ of\ paths\ of\ length\ 2'}$$

where a triangle is a set of three nodes in which each is linked by an edge with the other two.

Modularity (Q)

The optimal modular partition is a subdivision of the network that is able to maximize the ratio between intra-module edges vs inter-module edges (Newman, 2006). Q parameter is a statistic representing how well the partition of the current network fits that definition. We calculated modularity as the average Q through 100 runs considering that the value on each run may slightly vary due to heuristics in the algorithm. Q value was calculated according to Newman´s formula (Newman, 2006) by subtracting the expected number of edges in a given community from the actual number

of such edges. In a particular division of a network larger Q values indicate stronger community structure.

5.4.9. Nodal parameters

To best localize and define topological changes in the network we studied the alterations shown by MCI and SCD at the nodal level in three key parameters. For the sake of brevity, all the results at the nodal level correspond to an intermediate matrix density. (i.e. 15%), although several other thresholds were inspected to ensure the consistency of the results across different matrix densities.

Local clustering coefficient (C_{loc})

To further characterize differences in local connectedness in both groups we studied variations in clustering patterns across all 52 ROIs between groups with the above-mentioned clustering definition. However, instead of averaging the clustering coefficients across our 52 nodes, we inspected the clustering for each node separately.

Degree (D)

The degree is a measure reflecting the importance of a node in a network. It is calculated by counting the number of suprathreshold links in which a node is involved after binarization of the correlation matrix, that is to say, the number of neighbors a node has after thresholding.

Modular structure

Differences in modular structure were studied comparing partition assignments at the nodal level between groups. To this aim we calculated for each subject 1000 partitions and then selected the most representative subdivision of the network for each subject (Lancichinetti & Fortunato, 2012). After that, we employed the same procedure to choose the most representative partition for each group from the partitions of their members. In each step, we first calculated an agreement matrix with the *n* initial partitions (n = 1000 for each subject in the first step, and n = number of subjects in each group in the second step). This matrix contained the probability for each two nodes of the network of being included in the same module. After that, we employed the algorithm as described in ref (Lancichinetti & Fortunato, 2012) to find a consensus partition of the agreement matrix. This algorithm partitions the agreement matrix a number of times to then extract a single representative agreement partition.

5.4.10. Nodal parameters

We used ANCOVA with age as covariate to test group differences in all the network parameters ($\alpha = 0.05$). Besides, considering that MCI patients had

a lower educational level, we repeated all the analyses with education as a covariate and ensured that our results were not driven by this variable as all the effects reported remained unchanged. In the case of nodal degree and clustering, p-values were corrected using False Discovery Rate for each specific comparison (Benjamini & Hochberg, 1995) (Q = 0.2). To test differences in modular distribution at the node level, we calculated differences between each pair of partitions by means of their distance as an information theoretic measure as implemented in (Meilă, 2007). This procedure quantifies the normalized variation of information between partitions applying the equation:

$$VI_n = \frac{H(X) + H(Y) - 2(MI(X,Y)}{\log(n)}$$

where H is the entropy of each partition, MI is mutual information and n is the number of nodes. X and Y correspond to each of the modular partitions compared.

Intra-group and inter-group distances were compared to test for differences in modular structure between groups. Afterwards, we compared the intra-group variability of the individual partitions across groups with a Wilkoxon test.

We conducted correlation analyses using Pearson coefficient in our whole sample between our network parameters: (small-world, clustering, transitivity, modularity and individual partition distance to their group´s standard partition) and a subset of neuropsychological tests, one belonging to each of the cognitive domains mainly affected in early AD: memory with the "immediate recall test" from the Wechsler Memory Scale, and executive functions with the TMTb time. We also included MMSE as an indicator of overall cognitive status. With this analysis we aimed to study the relationship between network disruption and cognitive status. P-values for the correlations were also FDR corrected.

5.5. Results

5.5.1. Network results

Small-world (S)

Regarding small-world (Figure 5-1), all three groups exhibited small-world like brain network topologies with values over 1 for all matrix densities. MCI patients showed a decrease in most of the thresholds with respect to HC in the theta (p-range = 0.0001 - 0.044) and beta band (p-range = 0.00001 - 0.037). MCI also exhibited a decrease in S compared to SCD in theta (p-

range 0.02 - 0.045) and beta bands (p-range = 0.01 – 0.047). However, differences between MCI and SCD were significant for a fewer number of thresholds. There were no significant differences in small-world topology between HC and SCD groups.

Clustering (C_{glob})

MCI and SCD elders exhibited a decrease in global network clustering in theta and beta bands with respect to HC. SCD clustering values were intermediate between HC and MCI (Figure 5-1). More concretely, network clustering in MCI patients was decreased compared to HC in the theta (p-range = 0.0004 – 0.037) and beta band (p-range = 0.00003 – 0.049). There were no differences in clustering coefficient between MCI and SCD in theta or alpha band. Significant differences between these two groups were found only for two matrix densities in the beta band (p-range = 0.027 – 0.036). However given that these results are very sparse across thresholds they should be cautiously interpreted, as they could reflect an arbitrary effect due to the specific thresholding value. Similarly to MCI, SCD elders showed a decrease in the clustering coefficient with respect to HC in theta (p-range 0.018 – 0.04) and beta bands (p-range 0.015 – 0.045). However, these differences were evident in a smaller number of thresholds in both frequency bands.

Modularity (Q)

MCI patients exhibited increases in modularity in theta band when compared to HC (p-range = 0.001 – 0.049) and SCD (p-range = 0.022 – 0.041), although the latter comparison involved only 3 significant matrix densities. In this case differences between MCI and SCD should be carefully interpreted given that SCD showed intermediate modularity values between HC and MCI in this matrix density range (Figure 5-1). In the alpha band SCD network modularity was decreased compared to both HC (p-range = 0.022 – 0.048) and MCI (p-range = 0.022 – 0.048). Regarding beta band an increase in modularity was observed in MCI patients with respect to HC (p-range = 0.009 – 0.044) and SCD (p-range = 0.015 – 0.037).

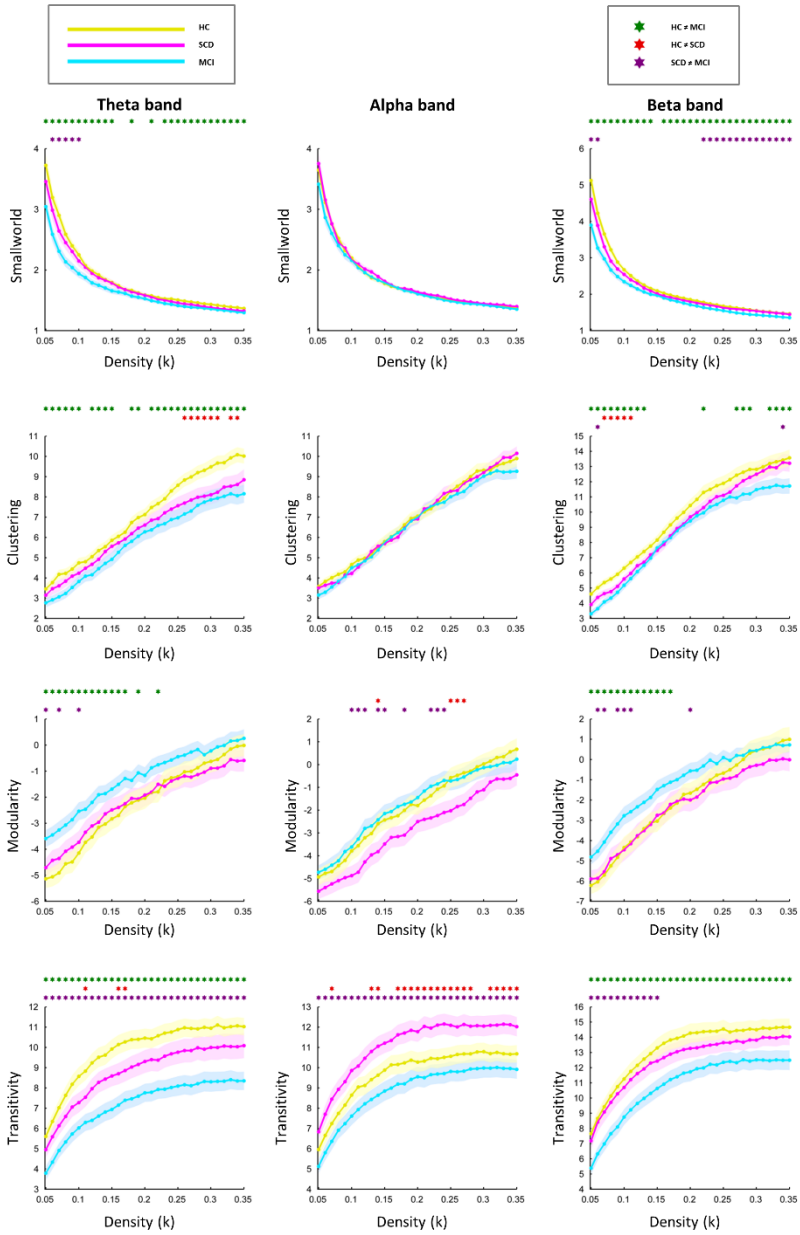

Figure 5-1. This Figure displays mean network parameters for each diagnostic group as a function of matrix density (k). Shaded areas represent mean standard error. If the difference between two groups for a given matrix density is significant (α=0.05), an asterisk with the specific color code of that comparison is shown in the upper part of each graph. Each row shows the results for a different network parameter: small-world, clustering, modularity and transitivity respectively. Each column corresponds to a specific frequency band: theta, alfa and beta band respectively.

Transitivity (T)

In the theta band we observed a progressive decrease in transitivity from HC to SCD and then MCI (Fig 1). Concretely, SCD transitivity value was significantly lower compared to HC in a few thresholds (p- range = 0.042 – 0.048), but still higher than MCI for every matrix density (p-range = 0.013 – 0.045). It is important to take into account that even though SCD transitivity in this frequency band showed an intermediate decrease between HC and MCI, the statistical significance was not robust across thresholds, so this result should be carefully interpreted. MCI transitivity was also decreased with respect to the HC group (p-range = $8 \cdot 10^{-6}$ – 0.0001). Network transitivity was increased in SCD elders in the alpha band with respect to both HC group (p-range = 0.016 – 0.048) and MCI patients (p-range = 0.0009 – 0.005). Regarding beta band, MCI patients exhibited a decrease in transitivity with respect to SCD (p-range = 0.005 – 0.044), and a more consistent decrease across thresholds when compared to HC group (p-range = 0.0004 – 0.03).

5.5.2. Node results

Local clustering changes

As shown in Figure 5-2, we observed alterations in clustering coefficient in the MCI group when compared to HC at the node level. In the theta band, these alterations involved clustering decreases specially located in posterior areas and lateral areas such as left middle temporal lobe, both inferior parietal lobes, and right superior occipital regions. Two frontal nodes also exhibited a decrease in clustering in this frequency band in MCI patients. Regarding beta band, we observed a posterior decrease in nodal clustering in MCI patients affecting inferior and middle temporal lobe and right lingual gyrus. However, we found an increase in clustering coefficient in the frontal lobe in both; right superior medial and left middle frontal lobe. Interestingly, SCD clustering levels seem to be in an intermediate state between HC and MCI as we found no differences between HC and SCD nor between SCD and MCI.

Local degree changes

Our results showed alterations in nodal degree both in SCD and MCI, although the latter showed disruptions over much broader regions (Fig 3). With respect to the HC group, MCI patients exhibited a clearly divided dual pattern of posterior degree decreases and anterior increases in theta and beta band. Posterior nodes affected by this degree decrease involved bilateral occipital, middle temporal lobe and parietal areas including two nodes located in the left precuneus. Regions with increased nodal degree included bilateral middle and superior frontal gyrus, left hippocampus, anterior cingulate cortex and the insula among others. SCD elders also exhibited a

nodal degree increase in left postcentral node with respect to HC in the beta band

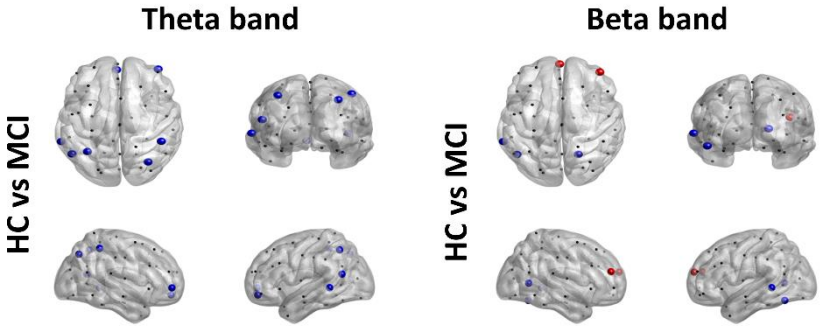

Figure 5-2. Figure displays significant nodal clustering differences between groups. Blue spheres indicate a decrease in the specific node in MCI group. Red spheres indicate an increase in the specific node in MCI group.

The comparison between SCD and MCI revealed similar results in the theta band, where MCI patients demonstrated lower degree values over posterior brain regions and degree increases in anterior nodes of the network. Similar regions were affected in the theta band in both comparisons (HC vs MCI and SCD vs MCI) although a smaller number of nodes showed degree abnormalities in the latter. Furthermore, in the alpha band one node located in the left superior occipital lobe showed a significant decrease in degree in the MCI group with respect to SCD. Interestingly, no differences were found between SCD and MCI in the beta band, in which MCI exhibited larger alterations with respect to the HC group

Modular distribution

We did not observe significant modular distribution changes between groups in any of the contrasts ($\alpha = 0.05$). That is to say, between-groups partitions distance was not significantly larger than the intra-group partitions distance. Given that we did not observe significant modular changes between groups only HC module partitions are shown in figure 4.

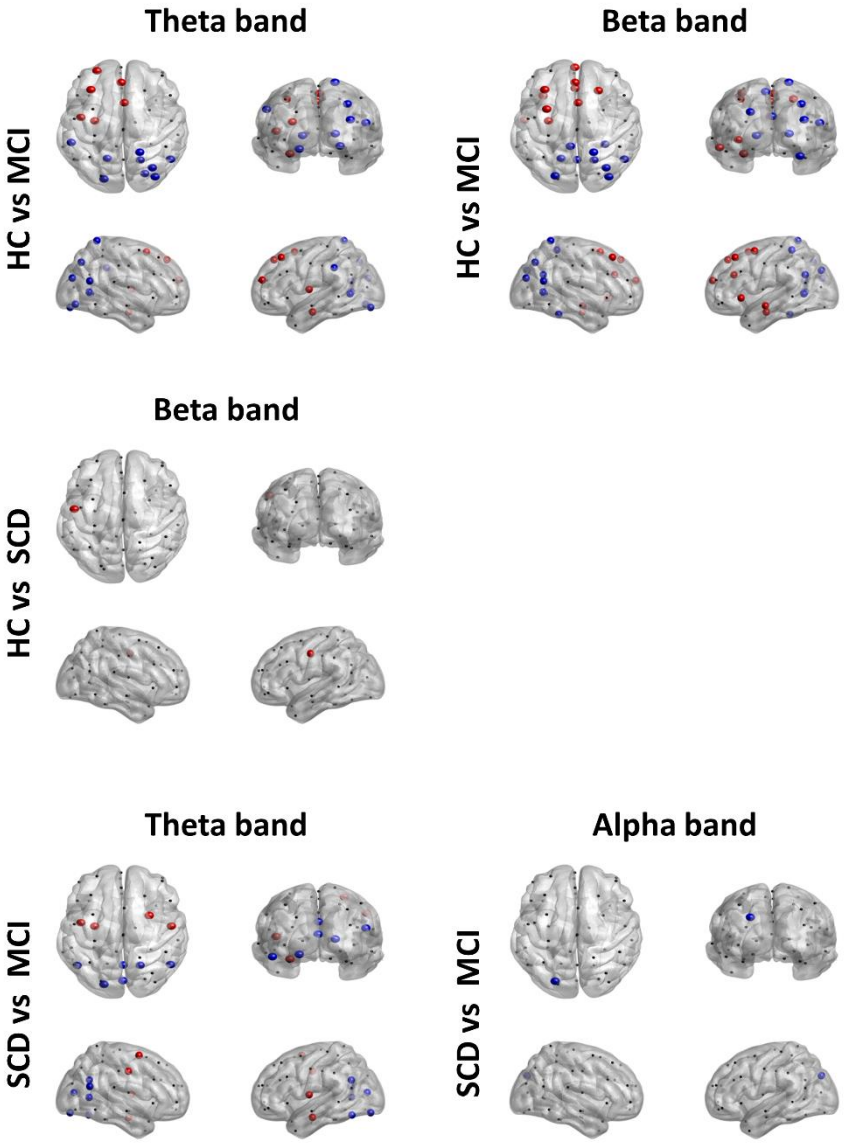

Figure 5-3. Figure displays significant nodal degree differences between groups. . Blue spheres indicate a degree decrease in the group in a more advanced stage of the disease (i.e. MCI<HC, SCD<HC and MCI<SCD respectively). On the contrary, red spheres indicate degree increases in this group (i.e. MCI>HC, SCD>HC and MCI>SCD respectively).

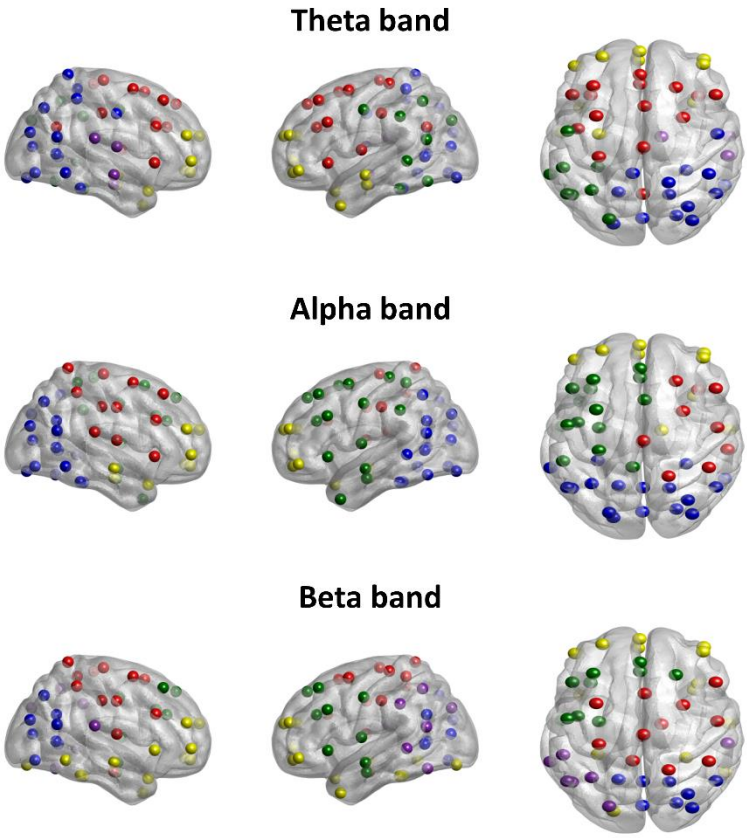

Figure 5-4. Figure displays modular partitions for HC group in in theta, alpha and beta band respectively. The color of each sphere indicates that node belongs to a specific brain module.

To further investigate modular distribution properties in each group, we studied in more detail the intra-group variability, comparing within-group partition distances across diagnostics *i.e.* the distance between modular partitions of each possible pair of participants in each group. Interestingly, we observed a highly significant linear increase in the intra-group modular variability with advancing pathology in the theta and beta band (Figure 5). Specifically, in the theta band MCI patients showed more variability than HC ($p < 0.001$) and SCD ($p < 0.001$). Furthermore, SCD modular partitions variability between subjects was also larger compared to HC ($p < 0.001$). Similarly, in the beta band, MCI showed the largest between subjects distances, with respect to both HC ($p < 0.001$) and SCD ($p < 0.001$). SCD variability was also larger compared to HC ($p = 0.014$). Lastly, MCI patients also presented larger intra-group distances in the alpha band than SCD ($p < 0.001$) and HC ($p < 0.001$). On the contrary, we did not observe

a linear increase in this specific frequency range as SCD elders demonstrated lower between-subject distances than the HC group ($p < 0.001$).

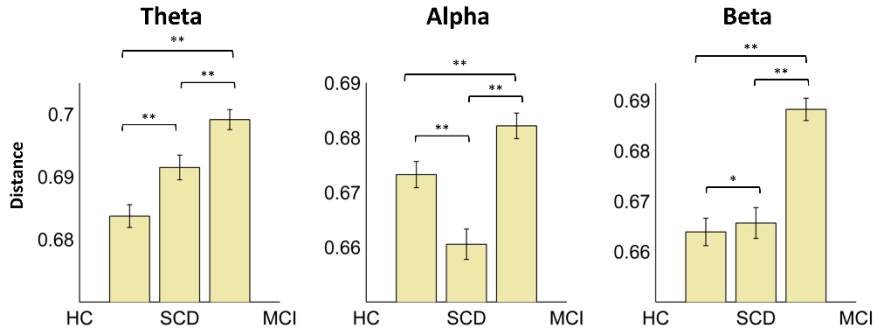

Figure 5-5. Figure displays the mean distance between module distributions of each pair of participants in each group in theta, alpha and beta bands (left to right respectively) and the standard error of the mean for each bar. * stands for $p < 0.05$. ** stands for $p < 0.001$.

5.5.3. Hippocampal volume

We compared hippocampal volume extracted from the MRI T1 images in our sample. We used an ANCOVA with diagnostic as the main factor and age as covariate. A significant main effect was found for diagnosis ($p < 10^{-6}$). Post-hoc comparisons revealed that MCI normalized hippocampal volume was significantly smaller compared to both HC ($p < 10^{-5}$) and SCD ($p < 10^{-4}$). Interestingly HC and SCD did not show any differences in their hippocampal volume ($\alpha = 0.05$).

5.5.4. Correlations

To help interpret the meaning of the disruptions found in SCD and MCI groups we conducted a series of correlations in our sample. The complete set of significant correlations after FDR correction is listed in Table 2. Regarding network parameters, we observed that in the theta, and beta bands, the disruptions observed in SCD and MCI correlated with a worst cognitive state. More specifically, decreases in theta or beta band in small-world or transitivity, and increases in modularity were associated with a poorer performance in neuropsychological assessment. Additionally, in the alpha band, a decrease in modularity and an increase in transitivity were associated with a higher MMSE score. No significant correlations were found between cognitive performance and clustering coefficient.

		THETA BAND		ALPHA BAND		BETA BAND	
Network	**Cognition**	rho	p-value	rho	p-value	rho	p-value
Small-World	*Imm. recall*	-	-	-	-	0.2	0.005
	TMTb time	-	-	-	-	-0.19	0.01
Modularity	*MMSE*	-	-	-0.16	0.03	-	-
	Imm. recall	-	-	-	-	-0.22	0.003
Transitivity	*MMSE*	0.19	0.009	0.14	0.04	-	-
	Imm. recall	0.2	0.007	-	-	0.31	$2 \cdot 10^{-5}$
	TMTb time	-0.17	0.03	-	-	-0.2	0.009
Partition deviation	*MMSE*	-	-	-	-	-0.15	0.03
	Imm. recall	-	-	-	-	-0.25	$5.5 \cdot 10^{-4}$
	TMTb time	-	-	0.18	0.01	0.23	0.002

Table 5-2. **Correlations between network metrics and neuropsychological performance.** Significant correlations and their p-values after FDR correction between cognitive tests and network parameters for each frequency band.

Lastly, in order to better characterize the changes demonstrated by SCD and MCI in the intra-group variability of their modular distributions, we conducted a series of correlations. For each subject, we obtained the distance between his own modular partition, and the representative partition of his group. Afterwards, we correlated that distances with neuropsychological tests. In the beta band, we observed that the more different a subject´s modular distribution was from his group, the worst his performance was in memory, executive functions and his general cognitive state. In the alpha band, where SCD showed a decrease in intra-group variability, we observed a similar pattern; those subjects with similar partitions to its group were able to complete TMTb faster than those with more different modular distribution.

5.6. Discussion

In the present work we found several alterations in the network organization of MCI and SCD elders. Elders with MCI exhibited decreased small-world, clustering and transitivity and increased modularity. Furthermore, we observed disruptions in the degree distribution of their nodes and in their nodal clustering. Lastly, the variability of their modular distributions was

significantly increased, thus exhibiting great disparity between subject´s partitions. However, the most striking and novel finding is the presence of alterations in several network parameters in healthy elders with SCD, such as clustering, transitivity, modularity and the changes observed in intra-group partitions´ heterogeneity. More importantly, despite the fact that these disruptions were less pronounced in SCD, the majority of these changes were in line with those found in MCI. SCD showed intermediate values between HC and MCI groups in multiple network parameters. This is the first study reporting network disruption in SCD elders. To the best of our knowledge, the only study to date studying network topology in SCD reported preserved white matter organization in SCD (X.-N. N. Wang et al., 2016). Interestingly, in their study SCD elders also exhibited intermediate network parameter values between HC and MCI. The larger sample size of our study probably allowed us to detect significant differences accounting for the discrepancies in the results. Our findings support the notion of SCD as an asymptomatic at-risk state of AD, suggesting an AD-continuum starting several years before clinical manifestations appearance in the MCI stage.

At the network level, we observed several alterations in the different parameters estimated in both MCI, and SCD. Interestingly, most of these disruptions were found only for the theta and beta bands, and not in the alpha range, which is the predominant resting state rhythm. This could suggest that network structure in the alpha band is relatively preserved until later in the course of the disease.

A loss of small-world like architecture has been traditionally associated with AD (Morabito et al., 2015; Sanz-Arigita et al., 2010b) and it has also been documented in MCI patients (Pereira et al., 2016). In our data, the reduction observed in small-world affected only MCI group in theta and beta bands. This decrease in small-world is thought to impair normal brain organization. Furthermore, MCI networks are known to be less efficient and slower in information flow (Ahmadlou, Adeli, Bajo, & Adeli, 2014), which is consistent with a decrease in smallworldness. Smallworld-like networks combine a high number of short range connections at a low cost, with some long range connections maintaining an efficient information flow between distant brain regions (Salvador et al., 2004). This specific network disorganization present in AD has been related with alterations in regions with a critical role for information flow, the so-called hubs (Stam et al., 2008), which are known to be affected in the early stages of the disease (Matthew R. Brier et al., 2014). However, small-world architecture seems to be preserved in the SCD stage.

Clustering and transitivity parameters reflect how well the different regions of the brain are connected into their own local cluster. Even though studies

reporting transitivity are yet scarce, previous results suggest that it is a superior method to study AD-like networks (Pereira et al., 2016). As a consequence, we calculated both parameters to ensure similar results and compare with previous literature, which mainly used clustering coefficient in AD. Overall, we observed decreases in local connectedness in MCI and SCD, specially affecting theta and beta bands. There are no previous studies reporting decreased clustering coefficient in SCD. However, a reduction in network clustering has been reported with different methods such as functional magnetic resonance imaging (fMRI) in AD (Matthew R. Brier et al., 2014; Supekar, Menon, Rubin, Musen, & Greicius, 2008) or cortical thickness and volume with MRI in MCI patients (Pereira et al., 2016) who also exhibited transitivity reductions in this study. MCI and AD patients also showed clustering reductions in a previous study using Sparse Inverse Covariance Estimation with positron emission tomography (PET) (Ortiz et al., 2015). Thus our results for SCD and MCI are consistent with previous literature. The increase in transitivity in the alpha band showed by SCD elders reflects an increase in the number of short range connections in this specific frequency range. Interestingly, we observed that those subjects with higher transitivity values in the alpha band demonstrated an overall better cognitive status. Although still tentative, this result could suggest a compensatory mechanism underpinning the normal cognitive performance of SCD elders in spite of their abnormal clustering in other frequency bands.

Regarding modularity, we again observed a similar pattern in theta and beta bands. MCI patients exhibited increased modularity in those bands with respect to both HC and SCD. Modularity is thought to represent the functional organization of the brain, and its value is large when the nodes of each module are strongly connected to their relatives, and weakly connected to other modules. Our results are consistent with previous studies reporting an increase in modularity in MCI and AD patients (Pereira et al., 2016), and also in Parkinson´s Disease patients with MCI at risk of developing dementia (Baggio et al., 2014). However, there are also inconsistent results, and some studies reported decreased modularity in AD (Matthew R. Brier et al., 2014). Our finding points to a worse communication between different functional modules, thus decreasing the ability of the different network components to share information and work together. This inefficient communication between brain modules has been previously linked with worse cognitive performance in AD patients (de Haan et al., 2012). Interestingly, in our results higher modularity scores in the beta band were related to worse memory performance in immediate recall, and in previous studies they were also associated with memory and visuospatial ability impairment (Baggio et al., 2014). These findings highlight the pathological nature of modularity alterations. In the alpha band, SCD elders were the only group showing alterations; a significant reduction in modularity. Lower modularity in the alpha band was associated

with a more preserved cognitive state, suggesting again a possible compensatory mechanism. Furthermore, a previous study found that increases in task demand were associated with reductions in the modularization of brain networks (Kitzbichler, Henson, Smith, Nathan, & Bullmore, 2011).. This effect was interpreted as an attempt to increase long-distance synchronization between different functional systems to overcome cognitive demands. In fact, those subjects with lower modularity values performed faster. These findings seem to support the compensatory interpretation of the alpha band results in SCD elders.

After studying network disruption in the preclinical stages of AD we aimed to localize changes within the brain. We first compared the degree distribution across brain regions between the three different groups. AD is known to affect the hub-like behavior of crucial nodes of the network (Matthew R. Brier et al., 2014), but very little is known about the preclinical asymptomatic stage of the disease. In our analysis, MCI patients exhibited nodal degree decreases over critical areas of the posterior default mode network (DMN) such as precuneus, parietal and middle temporal structures in the theta and beta bands when compared to HC. AD is a disconnection syndrome known to reduce functional connectivity over posterior brain regions (Damoiseaux et al., 2012; D. T. Jones et al., 2011). Interestingly, this connectivity alterations in the brain match with the spatiotemporal distribution pattern of Alzheimer´s pathophysiology (D. Jones et al., 2015). In addition, we observed increases in node degree over anterior regions including frontal areas, temporal structures and a central hub of the anterior DMN; the anterior cingulate cortex. These findings reinforce previous work reporting disrupted nodal strength in posterior and temporal regions of the brain, and increases over anterior areas (Engels et al., 2015; Huang et al., 2010; Yu et al., 2016), and expand them to the preclinical stages of the disease. Besides, these results are compatible with previous work from our group reporting a dual pattern change in the FC profile of both MCI and SCD showing anterior hypersynchronization and FC reduction over similar posterior brain regions (David López-Sanz et al., 2017). Interestingly, we did not find in the present work such a pattern in the nodal degree of SCD group, which could indicate that even though significant synchronization alterations are already present in this group, degree distribution seems to be still relatively preserved compared to MCI patients. Furthermore, SCD group exhibited increased nodal strength in a postcentral node in the theta band. Using graph theory approaches, degree increases of certain nodes have been also associated with a greater node vulnerability in different contexts such as World Wide Web, metabolic or social networks (Albert, Jeong, & Barabási, 2000). Interestingly, SCD group did not show any significant difference in the beta band when compared either to HC or MCI, thus showing intermediate values between both states.

Regarding nodal clustering changes it is worth noting that the only differences were found between HC and MCI. SCD local clustering was different neither from HC nor from MCI group. This raises the idea that SCD is situated in a somewhat intermediate state between both conditions hindering the identification of significant differences. MCI elders exhibited widespread clustering decreases indicative of local disconnection in those nodes. This is consistent with previous works in MCI´s structural networks (Pereira et al., 2016). Furthermore, in the beta band we found both clustering decreases located in posterior regions and nodes exhibiting increased clustering coefficient over frontal areas. This shift in local connectedness from posterior to anterior areas has been found mostly for high frequency bands, which could explain the different pattern obtained for theta and beta bands (Engels et al., 2015).

Regarding functional networks organization, we lastly compared the different modular partitions of the three diagnostic groups. Modular structure of the brain has been repeatedly associated with cognitive performance (Buldú et al., 2011; de Haan et al., 2012; Kitzbichler et al., 2011), thus becoming a relevant network feature to characterize the preclinical stages of AD. We did not observe a consistent reorganization in preclinical AD networks. However, we observed (specifically for theta and beta bands) an increasing variability between subjects´ modular partitions with advancing pathology, thus, in average, differences between each pair of modular partitions of SCD or MCI patients were larger than in the HC group. These findings could point towards pathological modular structure disorganization. It is worth noting that in all three bands, those subjects whose modular partition were further from their representative group partition, performed worse in memory, executive functioning and overall cognitive state as measured by MMSE. Although modular reorganization in AD has been reported previously (de Haan et al., 2012; Pereira et al., 2016), small sample sizes and the use of merely descriptive methods (rather than statistical comparison) may explain divergent results. Particularly, taking into account the apparent increased variability in AD network organization, sample size seems to be a crucial factor to make modular comparisons robust and reliable.

We also included a brief analysis of gray matter integrity in a key structure in AD progression, the hippocampus. As expected, according to multiple previous studies (Ma et al., 2016; Maass et al., 2017), MCI patients showed signs of atrophy over medial temporal regions. However, it is of note that we did not observe any sign of hippocampal atrophy in the SCD group as they showed almost identical levels of hippocampal volume compared to HC. The results to this regard are quite inconsistent in the literature, while some studies found a significant deterioration in this region (Perrotin et al., 2015) others reported no signs of gray matter loss (Tepest et al., 2008).

While hippocampal volume represents a useful tool in later stages of the disease, as it has proven to correctly classify MCI and AD patients (Zhou et al., 2014), it is relevant highlighting that according to our results MEG is able to capture subtle alterations in network organization even before standard MRI volumetric analysis are able to detect them as reflected in our results.

This study reinforces the idea of SCD as a preclinical asymptomatic stage of AD. While still preserving some intact network features, SCD elders evidenced disruptions at the network level compatible with those exhibited by MCI patients, although to a lower extent. Interestingly, SCD group showed changes in transitivity and modularity in the alpha band in opposite direction to those exhibited by MCI patients, which could be interpreted as a compensatory mechanism. These findings are in line with previous studies reporting intermediate but detectable pathological levels in healthy elders presenting cognitive concerns in different modalities such as: MEG power spectra (D. López-Sanz et al., 2016), gray matter atrophy (Peter et al., 2014; Striepens et al., 2010) or β-amyloid accumulation in AD-related areas (Perrotin, Mormino, Madison, Hayenga, & Jagust, 2012; Snitz et al., 2015). Furthermore, previous studies found that β-amyloid accumulation could reach a plateau before the onset of AD (Duara et al., 2015). Consequently, it is a likely hypothesis that those SCD elders that will eventually develop AD show some accumulation at this stage already, thus disrupting to some extent their network behavior. More research is needed to expand and replicate the knowledge of this preclinical stage. However, these results highlight the relevance of cognitive concerns in the clinical setting and point towards a continuum in the prodromic stages of the disease from healthy control to MCI or AD through SCD stage.

5.6.1. Limitations and future directions

This study has two main limitations. On the one hand, some of the network differences reported should be cautiously interpreted as mentioned before. Namely, differences between SCD and MCI in beta band clustering or theta band modularity, or those in theta band transitivity between HC and SCD are restricted to only a few thresholds, thus limiting the robustness of those results. This problem is inherently related with the second major limitation of this study, the fact that SCD is a quite heterogeneous entity, thus increasing the variability of our results. Even though there are multiple evidences reporting SCD elders are at an increased risk for developing AD and MCI (Jessen et al., 2014; Mitchell et al., 2014) and show increased levels of AD pathology (Striepens et al., 2010; Visser et al., 2009), the present study is cross-sectional and the exact fraction of the SCD cohort that will go on to develop AD is unknown. Our current definitions and limited understanding of SCD hamper our ability to discriminate those

individuals with AD pathology from those that will not develop the disease. Future next steps should involve Computer Aided Diagnosis (CAD) in the SCD stage. Previous work has used CAD on AD and MCI with either MRI (F J Martinez-Murcia, Gorriz, Ramirez, Ortiz, & For The Alzheimer's Disease Neuroimaging, 2016; Francisco J. Martinez-Murcia, Gorriz, Ramirez, & Ortiz, 2016; Ortiz, Munilla, Górriz, & Ramírez, 2016), or MEG (Amezquita-Sanchez, Adeli, & Adeli, 2016; López et al., 2014) successfully classifying and diagnosing at an individual level, for a review see (Mirzaei, Adeli, & Adeli, 2016). However, its use applied to earlier stages is very limited nowadays. A refinement in SCD selection criteria is needed to allow us employing such procedures to study the evolution of this at-risk of AD population.

Author contributions:

DLS conducted MEG recordings; DLS and PG carried out data analysis; DLS and PG wrote the manuscript; DLS prepared the figures; BA, MLD and RLH recruited the sample and carried out neuropsychological assessment; FM and RLH designed the experiment. All the authors read and corrected the manuscript.

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5.7. Summary of conclusions

1. The main conclusion drawn from this third experiment is that although subtle, SCD present some signs of network structure alterations and MCI patient exhibit marked network reorganization in comparison with HC elders, especially in the theta and beta bands. Most of the changes observed in SCD matched the expected direction observed in the MCI group, although usually to a lesser extent, thus showing intermediate values between HC and MCI for most metrics.

    a. Small-worldness was largely reduced in MCI patients in comparison with HC and although the effect size was smaller, small-worldness was also reduced when compared with SCD who showed normal values in this metric.

    b. Local connectedness was significantly affected in SCD and MCI (theta and beta bands), compared to HC. They both showed classical AD patterns of local disconnection.

    c. Modularity was unaffected in SCD elders. However, MCI showed increased values thus showing more segregated and less integrated networks, which is again consistent with the hypothesis of the disconnection syndrome.

    d. Interestingly, SCD opposite changes to those showed by MCI in the alpha band in local connectivity (transitivity) and modularity.

2. At the node level we were able to find clustering decreases only in the MCI group. In the theta band, decreases of nodal clustering were found over widespread regions involving parietal, temporal, occipital and frontal regions. Interestingly, this pattern was different in the beta band where anterior increases in local connectedness were accompanied with posterior decreases in this same metric. This dual pattern was also present when assessing nodal degree reorganization in MCI networks in the theta and beta bands. SCD showed degree increases over left postcentral regions when compared to HC.

3. It is worth highlighting that, even though the representative modular partition in HC; SCD and MCI were comparable, we observed a significant increase in the variability between groups in the theta and beta bands that increased linearly with increasing AD pathology (i.e. HC < SCD < MCI). A possible progressive disorganization in AD brain networks is hypothesized.

Interestingly, SCD again exhibited an inverse pattern in the alpha band with decreased variability.

4. All the observed network alterations in theta and beta bands demonstrated to negatively affect cognitive performance. Of note, the changes observed in the alpha band in the SCD group could possibly represent a compensatory mechanism, as they predicted a better cognitive performance in the population.

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6. General discussion

This thesis comprises three different experiments carried out in the context of preclinical AD using neuroimaging techniques such as MEG and MRI. These studies aimed to elucidate the integrity of synaptic activity and brain networks in healthy elders with SCD and elderly in a well-established prodromal state of AD; MCI. Both groups were compared to a sample of healthy elders without cognitive concerns or impairment. All the experiments included in this work pursued a common goal, the description and comparison of brain functioning at these different pathological stages, thought to represent a hypothetical continuum towards AD dementia development.

Neuroimaging has been largely used to unravel brain changes within demented population (Risacher & Saykin, 2013) and in MCI patients (Lan et al., 2017). Nevertheless, previous knowledge about brain state in SCD stage is rather scarce, even though during the last few years the field has experienced a considerable growth and has received increasing attention and research efforts. As a consequence, the International Working Group (IWG) has recently proposed the use of electrophysiological techniques such as EEG and MEG to characterize the early dysfunctions occurring in the preclinical stages of AD (Dubois et al., 2016), based on the great sensitivity of these particular imaging modalities to detect subtle early signs of synaptic alterations.

In this context, the present thesis was able to characterize significant alterations in the electromagnetic brain activity of both SCD and MCI groups during resting state. First, a significant deterioration of the ongoing oscillatory activity of MCI and SCD was evidenced. By comparing the spectral properties in these two groups we observed an important relative alpha power diminish in both groups compared to HC. Decreases of alpha power have been traditionally reported in dementia patients (Babiloni et al., 2009) and they are known to be tightly related to cognitive deterioration in this population (Kuskowski, Mortimer, Morley, Malone, & Okaya, 1993). Our results are consistent with more recent literature extending these findings to the MCI stage (López, Cuesta, et al., 2014). However, the most relevant finding of this concrete experiment is the finding that SCD elders also showed a similar pattern of alpha relative power decrease. Alpha power decreases were found over bilateral prefrontal, occipital and temporal regions in the MCI group. Interestingly, although SCD elders exhibited alpha reduction over widespread regions, the areas showing a higher disruption were similar to those affected in MCI. Furthermore, the alpha peak frequency was significantly reduced in the MCI sample, in agreement

with previous findings (Garcés et al., 2013), but unaltered in the SCD group. This temporal progression of alpha power disruption suggests that once exceeded a certain pathological threshold, neural networks are no longer able to oscillate at the same frequency, and alpha frequency shifts towards slower frequencies.

Three possible mechanisms could underlie these results. Firstly, the generation of alpha (and other brain rhythms) is known to depend on cortico-thalamo-cortical loops resonating across the brain (Hindriks & van Putten, 2013). These loops rely on a large number of axon fibers connecting cortical regions with the thalamus back and forth. The diffusive properties of the networks in MCI patients are known to be affected showing disconnection signs similar to those exhibited by AD patients (Garcés et al., 2014; Teipel et al., 2014). Similar, but more restricted changes have also been reported in SCD elders which could support this hypothesis for alpha power disruption (Li et al., 2016). Second, Aβ accumulations which are known to affect both SCD and MCI (Chételat et al., 2010) have been related to dysregulation in the inhibitory system of the brain affecting specific GABAergic neurons (Garcia-Marin, 2009). This would eventually lead to an upregulation and disorganization of the firing rate due to inhibitory deficits. This desynchronized upregulated firing rate is known to produce decreases in alpha amplitude (Hanslmayr, Staresina, & Bowman, 2016; Klimesch, 2012). The third possible hypothesis is that alpha power decreases could be related to neuronal death which would reduce the amplitude of the resulting oscillation. This hypothesis is supported by the notion that Aβ accumulations are known to produce cell death (Jana & Pahan, 2010). However, despite the fact that MCI patients showed significant hippocampal atrophy, we did not observe significant gray matter atrophy in the SCD group, at least in this specific brain region.

After characterizing the spectral properties of the alpha rhythm, a subsequent study addressing the long-range synchronization in this frequency band was carried out. To tackle this issue a phase-synchronization index, named PLV was calculated to estimate the functional coupling between each pair of brain regions. An interesting pattern showing mixed hypo and hyper-synchronization emerged from comparing the three groups. We found very remarkable similarities between the FC disruption patterns exhibited by both SCD and MCI groups. These alterations included increased FC values over anterior regions, involving medial frontal as well as temporal structures, and decreased synchronization over posterior regions affecting occipital, postero-parietal and temporal cortices. These results are in high agreement with previous literature in AD patients using fMRI (D. Jones et al., 2015; Supekar, Menon, Rubin, Musen, & Greicius, 2008) and electrophysiological techniques (Yu et al., 2017). Again, the crucial added value of this study is

expanding this knowledge to the preclinical stages of AD, and in particular to SCD field, where no previous study had addressed resting state connectivity with EEG/MEG. Previous work suggested that hypersynchronization over anterior regions takes place early in the course of the disease (Damoiseaux, Prater, Miller, & Greicius, 2012). The second experiment was able to confirm this hypothesis, and to elucidate that SCD could likely represent a very early stage of AD in which initial FC alterations already exist (Damoiseaux et al., 2012). Our results also demonstrated that anterior hypersynchronization remained constant between SCD and MCI, desynchronization over posterior regions was further accentuated in this transition, which is in agreement with the prevailing disconnection hypothesis in AD (Bokde, Ewers, & Hampel, 2009; Delbeuck, Linden, & Collette, 2003). The intrinsic connectivity of different RSNs was compared between groups. We observed that MCI FC over pDMN and DAN were reduced compared to control, which has been related in previous literature to cognitive deficits (Fox et al., 2005; D. T. Jones et al., 2011). Of note, SCD patients showed decreased FC values in these same networks to a similar extent than MCI patients.

The etiopathogenic substrate of FC alterations has been largely related to Aβ accumulations, which are known to impair synaptic transmission (Shankar et al., 2007). In fact, the topographical distribution of Aβ deposition is tightly related to FC alterations (Myers et al., 2014), particularly in regions involved in the pDMN such as precuneus. Hyper-synchronization has been traditionally interpreted as a compensatory mechanism of the brain networks to maintain cognitive performance (Mormino et al., 2011). However, the direction of our correlations revealing a worse cognitive status in those subjects with higher FC and recent studies highlighting an increased risk of conversion for MCI patients exhibiting this pattern of hyper-synchronization (López, Bruña, et al., 2014) led us to interpret it as a pathological sign of the disease

After studying the dynamic properties of brain networks through FC analysis the last experiment of the thesis aimed at assessing network topology and structure at a more global scale. Network properties are known to ultimately underlie successful human cognition (Sporns, Chialvo, Kaiser, & Hilgetag, 2004) which concedes an extraordinary relevance to the study and understanding of these brain features. This third experiment included the largest sample employed in the thesis with a total of 187 participants divided into HC, SCD and MCI groups to compare network architecture in the theta, alpha and beta bands.

In this experiment a significant network disruption was evident in the MCI group, similarly to previous results on AD patients (Supekar et al., 2008). The most interesting finding of this experiment was that SCD elders showed

signs of network disruption similar to those present in MCI patients. However, despite exhibiting intermediate values in most of the metrics between HC and MCI, the SCD group showed little or no differences when compared with both groups. In particular SCD networks had lower clustering and transitivity values mainly in the theta and beta bands compared to HC. This pattern was accentuated in the MCI group and is interpreted as a loss of short range connectivity between neighbor nodes (Rubinov & Sporns, 2010). This finding has been also found in more advanced stages of the disease using structural networks (Pereira et al., 2016). This suggests that functional alterations in the network topology are present very early in the disease, and resemble the structural and functional alterations previously characterized in the dementia stage. Smallworldness, a measure of the balance between short and long-range connections (Vecchio et al., 2017), was reduced in the MCI group but preserved in SCD patients. This network property is known to capture individual cognitive abilities (Liao, Vasilakos, & He, 2017) and its deterioration in the MCI population could potentially underlie their cognitive worsening. The last network parameter considered was modularity, and was found to be augmented in the MCI group in the theta and beta bands and reduced in the SCD group in the alpha band. Increases in modularity in MCI can be interpreted as a reduction of the functional integration among brain regions in this group (Rubinov & Sporns, 2010) and is again consistent with previous literature reporting similar changes in MCI and AD in structural brain networks (Pereira et al., 2016). SCD modularity changes in the alpha band were interpreted as possible compensatory mechanisms as explained below.

Although far less exhaustive, we also carried out a simple comparison of the structural brain integrity across the different stages of preclinical AD. Notwithstanding, we only compared gray matter volume of a single cortical region: the hippocampus. This region is known to be closely linked to early pathology deposition in AD (Duyckaerts, Delatour, & Potier, 2009) and shows early atrophy signs even in preclinical stages such as MCI (Pihlajamäki, Jauhiainen, & Soininen, 2009). Although included in all the experiments, it is important to note that hippocampal volume analyses were equal in experiments 1 and 2 as they included the same sample. Experiment 3, on the contrary, included a larger sample, although results remained unchanged. In our studies we were able to replicate the previous literature findings: MCI patients showed lower normalized hippocampal volumes than HC. In fact, this classical sign of AD-related pathology accumulation was related with some of the abnormal MEG patterns described in this thesis such as lower individual alpha peak frequency or increased posterior hypo-synchronization. Furthermore, hippocampal volume is known to be associated with cognitive state as well as future decline (Bangen et al., 2017). Nonetheless, SCD elders did not show any sign of hippocampal

atrophy, despite considerable brain activity disruption. This fact remarks that MEG is able to detect subtle synaptic changes in taking place very early in the AD pathology, before hippocampal atrophy can be measured with MRI. Alternatively, it has been proposed that the use of MRI scanners with increased resolution and/or the use of more refined pipelines such as manual delineation could detect gray matter disruption in SCD, as very subtle alterations can go unnoticed otherwise (Perrotin et al., 2015).

6.1. Bridging the gap among experiments: an integrative perspective

In this thesis the electrophysiological properties of the brain activity was assessed at various stages of the preclinical AD. A progressive disruption of their local synchronization (i.e. power) in the alpha band, and alterations in long range-connectivity in the alpha band and global network structure in theta, alpha and beta were found relevant. Interestingly, these alterations were stronger in advancing stages of the disease. However, the relationship and implications between the results obtained in the three experiments carried out for this thesis is not trivial, and should be carefully addressed.

The first two studies presented characterized brain activity in the alpha band by comparing first their spectral properties (relative power and peak frequency) and their long-range synchronization (PLV in the source space). Alpha is the main rhythm in the human resting state and has been traditionally associated with top-down control by inhibitory processes (Jensen & Mazaheri, 2010). Previous work has shown that an increase in alpha power in a particular region is accompanied by a decrease in the firing rate and overall neuronal activity in that same area (Haegens, Nácher, Luna, Romo, & Jensen, 2011). Interestingly, this inhibitory control of neuronal spiking seems to behave cyclically, with its maximum coinciding with the peak of the alpha rhythm, approximately each 100 ms. The finding of reduced alpha relative power in both SCD and MCI could point to a deficit in inhibitory control over downstream cortical networks, which could in turn disrupt FC in these networks, as observed in experiment 2. A second line of research posits that alpha oscillations hold tonic alertness and highlights the relationship between alpha power and synchronization over widespread brain regions (Sadaghiani et al., 2010). In this vein, decreases in alpha power would be associated with decreases in the general alertness of a subject by modulations in the synchronization of specific networks. In recent work from the same group (Sadaghiani & Kleinschmidt, 2016) a relationship between local alpha power and long-range cortical synchronization is proposed. According to their model, alpha oscillations would be modulated by different RSNs. More concretely, FC in the cingulo-opercular (CO) network would enhance the amplitude of alpha oscillations

to maintain tonic alertness, DAN would be in charge of local reductions of the alpha power in task-related regions to allow cognitive processing while the fronto-parietal network (FPN) would control long-range phase synchronization ensuring a hierarchical and distributed processing. The findings in the first two experiments could, in fact, be linked according to this theory. The deficits observed in long-range synchronization, notably over regions belonging to the DAN, could set the basis for local desynchronization and the observed relative alpha power decrease. Future studies should address the specific relationship between these two features of the alpha rhythm (power and connectivity) using MEG, and focusing on the above-mentioned RSNs.

A capital objective of this thesis is the appraisal of network behavior throughout the different stages of AD. While using different approaches and methodologies, experiments 2 and 3 are devoted to this common purpose. In the former, the integrity of whole brain connectivity as well as the mean FC in two key RSN for AD development, such as DMN and DAN, were measured in two prodromal stages. In the last study of this thesis, network functional organization was assessed. Whole brain FC analyses revealed a common pattern of posterior hypo-synchronization and anterior hyper-synchronization. This pattern was significant and very similar in terms of spatial distribution in both SCD and MCI. This result indicates an early FC reorganization in the course of the disease. Interestingly, a very similar pattern was observed in the reorganization of network degree in the third experiment. In this study, only MCI showed a clear pattern of network weight redistribution, although SCD showed degree increases over postcentral areas. Interestingly, SCD showed a similar trend to that exhibited by MCI, although it was not significant with a $p<0.05$ threshold after correction for multiple comparisons and was therefore not reported in the original paper. Even though these results could be misinterpreted as redundant, they indicate different aspects of network behavior. FC estimates the statistical relationship between two brain regions as a measure of their coupling (Friston, 2011). Thus, the second experiment informed us that anterior regions of the brain are more strongly coupled to each other in SCD and MCI than in HC and pinpointed the specific brain regions involved. On the contrary, the centrality of a node indicates the relative importance of a given region: its weight within the whole network (Rubinov & Sporns, 2010). Network results revealed that although anterior regions may be already hyper-synchronized in the SCD stage with minor changes in degree centrality, it is in the MCI stage when severe network weight reorganization takes place.

These changes are consistent with previous literature indicating a shift from posterior to anterior in the localization of brain hubs (Engels et al., 2015). Posterior brain regions, such as precuneus or PCC, act as fundamental hubs

in healthy brain communication (Brier et al., 2014) and they are known to be severely affected in AD, therefore having a milder contribution to network information flow (Greicius, Srivastava, Reiss, & Menon, 2004). The results of both experiments are consistent with the hub vulnerability hypothesis (de Haan, Mott, van Straaten, Scheltens, & Stam, 2012). According to this model, regions acting as main hubs of network information flow face higher activity demands, leading to increased Aβ accumulation and oxidative stress that ultimately would induce region disruption and disconnection. This is in agreement with studies reporting a similar progression of Aβ deposits and FC alterations (Drzezga et al., 2011). Furthermore, the initial hyper-synchronization over anterior brain regions, increased degree centrality and clustering coefficient would predict in this framework additional pathological accumulation leading to a subsequent decline in FC and disconnection, which has been reported in advanced AD (Damoiseaux et al., 2012).

One of the main possible concerns of the results reported in the three experiments is the apparent lack of consistency between the bands affected in each experiment. While in experiment 1 and 2 alpha band showed clear signs of alterations, network alterations in theta and beta band were found in experiment 3. First, it is important to remark that the different analysis pipelines we employed in each experiment (i.e. spectral analysis, direct FC analysis and network analysis) capture different aspects of brain activity. Although they are probably related (Sadaghiani & Kleinschmidt, 2016), they are not expected to behave uniformly showing identical patterns. Brain rhythms are known to serve different processes (Buzsáki, 2006) and even to connect brain regions with specific patterns of information-flow (Hillebrand et al., 2016), thus challenging result interpretation. Since Berger´s discovery, alpha rhythm has been known as the strongest component of the spontaneous human electromagnetic activity. According to multiple studies, alpha plays a critical role in brain maturing, sensory and vegetative functions and particularly in impairment and cognition (for a review see, Başar, 2012). From this perspective, it could appear reasonable that the brain network properties (the highest level of analysis employed in this thesis) are relatively preserved in the very early stages of AD, i.e. SCD stage. However, there are at least two possible limitations inherent to the experiments design that should be taken into account. Firstly, the frequency range employed to define band limits was slightly shifted from experiments I and II (defined from the individual alpha frequency) to experiment III (classical band limits). The alpha range defined in experiments I and II (6.9 – 11.4 Hz) partially overlapped with the classical theta band (4-8 Hz), hence it is a reasonable assumption that the theta band considered in experiment 3 could contain some activity which was considered as alpha in experiments 1 and 2. Secondly, different FC metrics were employed for experiments 2 and 3. While phase synchronization was used to quantify FC in experiment

2, a leakage corrected amplitude synchronization metric was used in experiment 3, which is better suited for brain networks metric. The choice of the appropriate FC metric is not trivial and could have a significant impact on the results (Lithari et al., 2012).

6.2. Electrophysiological brain changes and cognition in SCD and MCI

The major finding of this thesis is that brain functioning of healthy elderly individuals with preserved cognitive performance shows different patterns depending on whether they have memory concerns, at least at a group level. These results could potentially explain why SCD elders are known to be at an increased risk of AD (Fernández-Blázquez, Ávila-Villanueva, Maestú, & Medina, 2016). Even though their cognitive functioning is still comparable to that shown by HC elders, the integrity and behavior of their local and distant network seems to be at an intermediate state between both HC and MCI patients. This apparent dissociation between brain state and cognitive performance in the SCD group might not truly represent an inconsistency. There are three different possible explanations to this finding. These three options are not necessarily exclusive; on the contrary, they could represent complementary mechanisms underlying the relationship between brain state and cognitive performance in aging, and more concretely in SCD.

1. The threshold theory is probably one of the earliest interpretations given to discrepancies observed between brain health and clinical status. According to this hypothesis (Satz, 1993), there is a given amount of brain damage or pathology for each individual that can be absorbed without any visible clinical manifestations. In the described experiments we observed an alpha relative power reduction, antero-posterior patterns of FC disruptions and subtle network alterations. According to this theory, SCD alterations would not reach the threshold necessary to trigger cognitive impairment. Consistently with this theory, in all the studies, larger alterations were observed in the MCI patients, who exhibited not only power decreases but also slowing of the alpha peak, further posterior desynchronization and generalized brain network alterations. Interestingly, some of the changes that affected only MCI patients, such as alpha peak slowing, predicted steeper cognitive decline, reinforcing the idea that probably the amount of burden observed within the SCD sample is not enough to produce a significant cognitive impairment.

2. A second possible mechanism underlying the apparent discrepancies between observed brain impairment and the absence

of cognitive deficits may lay in compensatory mechanisms. According to Stern (2012), passive mechanisms establishing a fixed threshold of brain reserve, are not enough to explain the overwhelming complexity of brain networks. In this vein, there may be compensatory mechanisms through which brain networks affected by certain pathology might cope with that burden by recruiting additional networks or by reshaping its architecture. This approach would not be focused on what is lost but on what is left regarding brain health (Stern, 2002). The results of the third experiment, characterizing structure of functional brain networks in the three groups could support this interpretation. SCD elders exhibited certain restructuring in the alpha band, more concretely their networks were less modular and showed higher transitivity than those of HC. Interestingly, these changes were in opposite direction to those exhibited by the MCI patients. Modular structure is the cornerstone of the balance between brain segregation and brain integration, a critical aspect of cognitive performance (Friston, 2011). Lower modularity values in the SCD group represent a more integrated network with a lower degree of functional partitioning, which in our analysis was correlated with an overall better cognitive state as reflected by higher scores in the MMSE. The increase in transitivity highlights a local connectedness boost that was again related to better cognitive status.

3. A third possibility is that SCD elders, although indistinguishable from HC in neuropsychological evaluations, could in fact exhibit meager cognitive deficits. Current neuropsychological assessment tools show good sensitivity to detect the typical pathologies they were built for. However, it is plausible that SCD elders already present a slight decrease in their cognitive performance with respect to their previous level that still lies within the normal range, thus remaining undetected. This interpretation is supported by previous work reporting slightly worse cognitive performance in the SCD group, that, although undetectable at an individual level, is highlighted as a group trend (Caramelli & Beato, 2008; Minett, Da Silva, Ortiz, & Bertolucci, 2008; Prichep et al., 2006). Furthermore the hypothesis that subtle cognitive changes might be already present in the SCD stage is reinforced by a recent work reporting relatively large effect sizes in a specific task of feature binding assessing short term visual memory (Koppara et al., 2015).

It is important to bear in mind that throughout the three experiments presented in this volume generalized negative associations between the observed changes in SCD and MCI and their cognitive performance were

observed. These findings underscore that there is a progressive and slow disruption affecting brain networks much earlier than AD dementia can be diagnosed. Although here we focused on brain function, structural networks seem to be affected relatively early in the disease as well (Fischer, Wolf, Scheurich, & Fellgiebel, 2015). Most importantly, our correlations between brain function and cognitive scores point out that the above-discussed changes observed in our population could form the basis for subsequent cognitive deterioration, as recent studies highlighted an increased risk for cognitive decline in SCD (Dardenne et al., 2017).

7. Final conclusions

The results of three different experiments have been presented in this original thesis. In each of them, the state of brain activity as measured with MEG was assessed in three groups: a healthy control group of elders (HC), a group with healthy elders with significant cognitive concerns (SCD) and a group of elders with MCI. After presenting and discussing each specific finding, the main set of conclusions that can be drawn from this work are listed hereunder:

- Healthy elders presenting a significant level of cognitive concerns without objective detectable impairment show a significant level of brain disruption. This thesis demonstrated that even after ruling out major confounders such as depression, which has been reported to interact with cognitive complaints, the group of SCD elders presented electrophysiological abnormalities. These results remark the importance of considering SCD as a relevant clinical entity that should be considered beyond merely personality trait.

- SCD elders presented alterations in their MEG power spectrum. They exhibited alpha relative power decreases over widespread brain regions, specially marked over orbitofrontal and occipital areas.

- The normal pattern of brain FC is disrupted in the alpha band in the group of SCD elders. They showed a mixed topology of alterations combining hypo and hyper-synchronization. Hypo-synchronization affected posterior regions involving occipital, parietal and temporal areas mainly. Brain regions showing increased FC were mainly limited to the anterior parts of the brain such as anterior cingulate, or inferior frontal gyrus among others.

- Network functional organization and topology is slightly impaired in SCD elders. They showed reduced local connectedness, as reflected by decreases in both clustering and transitivity. Furthermore, their modular structure was found to be significantly more variable than those of HC elders. This latter finding could represent an early indicator of network disorganization known to affect AD patients in more advanced stages. Furthermore, SCD elders showed signs of compensatory changes in their network properties (global modularity and modular partitions variability) in the alpha band. These results could potentially underlie their preserved cognitive status.

- SCD elders do not show any detectable sign of objective cognitive decline at the individual level compared to HC that allows their diagnosis as MCI or pre-dementia patients. However, even within the range of normal cognition for their age, they might show minor changes in their performance. This could be supported by the significant group differences observed between SCD and HC in immediate and delayed recall performance of the WMS-III scale.

- Electrophysiological changes in both SCD and MCI, even in resting state, seem to have functional implications for their cognitive capacities, as revealed by correlational analyses carried out in the three experiments. Overall, negative associations were found between the observed changes and cognitive performances in classical neuropsychological tasks, in a way that those SCD and MCI individuals showing stronger electrophysiological alterations compared to HC (whether it is a power estimate, FC value or a network metric) showed worse cognitive performance.

- Electrophysiological changes appeared at an earlier stage than typical structural alterations. Although SCD elders exhibited significant MEG activity disruption when compared with HC, they did not show any sign of hippocampal atrophy. On the contrary, MCI patients showed both alterations. This finding could indicate that synaptic alterations appear before any anatomical alteration can be detected using typical volumetric hippocampal MRI, and highlight the utility of MEG in the context of early synaptic malfunctioning detection.

- A major objective of this thesis was to evaluate if SCD presented any electrophysiological alteration. But beyond that, the main objective was to assess whether those alterations could be associated with typical AD pathology signs that could support the inclusion of SCD as an early marker of AD. The changes observed in SCD resembled those found in MCI in the three studies. Interestingly, most of the alterations observed in SCD followed a similar trajectory than those of MCI, but to a lesser extent. These findings suggest that SCD could in fact represent an initial step in the transition between a completely healthy state and the slight but detectable cognitive deficit observed in MCI.

- MCI typical alterations such as alpha power reductions, slowing of their power-spectra and FC alterations are replicated in this thesis. Furthermore, valuable information about their network structure disruption, and the specific frequency bands and metrics affected is added by this work.

- This thesis described for the first time the specific evolution of different electrophysiological brain alterations known to be signature changes in AD pathology.
    - Regarding spectral changes in the alpha band in AD, there is an initial decrease in alpha power which is already present in SCD. This alpha power decrease is maintained towards the MCI stage coupled with the characteristic slowing of its peak known to affect AD patients and leading to the well-known "shift-to-the-left" of the AD dementia power-spectrum.
    - FC alterations emerge at an early stage of the disease with increased FC over anterior regions present in SCD elders. This hyper-synchrony is maintained at a similar level in MCI. Posterior disconnection is already significant in the SCD stage but these alterations seem to worsen with advancing pathology, as they are more pronounced in MCI.
    - Network alterations are already present in the SCD stage, although they are restricted to specific network parameters such as clustering and do not affect broad brain regions. It is in the MCI stage when major network alterations take place affecting multiple brain features and widespread areas of the brain.
- The final remark that can be extracted after considering all the above-mentioned conclusions is that cognitive concerns could and should be taken into account in the clinical setting. Dementia is an overwhelming disease that carries with it an immense amount of personal, social and economic costs. The correct detection and classification of cognitive concerns according to the criteria and guidelines established led us to the identification of significant brain abnormalities. These findings can be translated into the clinical context to promote early interventions aimed at reducing or slowing the appearance of cognitive symptoms that ultimately lead to disability and dependency in demented patients.

8. Limitations and future directions

The experiments described in this thesis represent a step forward in our knowledge about the electrophysiological basis underlying preclinical AD stages. However, they are not devoid of certain limitations that could not be addressed in the present work. In the following lines, main questions that should be answered in the following years by upcoming research are proposed. Furthermore, methodological considerations that should be taken into account in future literature are also mentioned.

- Replication is a fundamental step that should be prioritized in order to warrant the quality and faithfulness of each new result. However, recent trends governing publishing policies dramatically decrease the likelihood of replication studies being published. Nonetheless, it is fundamental verifying the findings reported in this thesis in future studies, as most of the analyses presented in the three experiments had never been previously reported in SCD population. Sample sizes used in this work are enough to assure a sufficient statistical power, particularly in the context of neuroimaging research. However, replications in independent and/or larger samples are necessary. Lastly, as previously discussed, analysis pipeline choices such as FC algorithms and graph metrics affect the results; hence replication with different imaging modalities and methodologies becomes crucial.

- This thesis describes different spectral, connectivity and network patterns characteristic of the SCD and MCI stage. Nonetheless, it is very important carrying out follow-up studies in these populations to understand the clinical significance of those alterations. Follow-up studies should assess the individual neuropsychological trajectory as well as the evolution of their brain electromagnetic profile, in order to detect as soon as possible the onset of objective cognitive impairment in the current SCD group and dementia in our current MCI patients. Tracking the specific patterns able to predict higher conversion rates to MCI and dementia is critical to bring this work to fruition and should certainly be considered in future work.

- One of the main limitations of the present work is the absence of biomarkers of Aβ or tau pathology in our sample. Brain accumulation of these proteins, or their concentration in CSF are known to increase the reliability of AD diagnosis and prediction. Additionally, it has been suggested to include those biomarkers in

the set of inclusion criteria of MCI (Albert et al., 2011) and SCD (Jessen et al., 2014) in order to increase the likelihood of their symptoms truly representing an early AD process. In the future, the replication of the present studies should be considered, improving sample recruitment with the addition of Aβ or tau biomarkers. However, it should be remarked that such biomarkers come at a high cost. Aβ and/or tau accumulation estimates can be obtained by either acquiring a PET scan with the corresponding ligand for each subject, which implies a high economic charge, (and the use of ionizing radiation), or by analyzing CSF for what a lumbar puncture is needed, thus hindering ethic approval and sample recruitment.

- The present thesis serves as a first step in the characterization and description of a largely unknown entity as is SCD in the aging process. As such, it is focused on group level statistics, to analyze and identify patterns of alterations that can help us unraveling the early process of the disease when cognitive symptoms are yet undetectable. However, a future line of work should aim at the individual diagnostic, trying to detect and replicate group level patterns on those individuals at risk of AD. This level of analysis is far more complicated, given the high variability among SCD sample. Particularly considering that only a portion of them will develop any type of dementia in the near future. However, with the inclusion of Aβ or tau biomarkers or considering genetic factors such as the presence of APOEε4 it could be possible to decrease sample variability, thus promoting these analyses. Furthermore, the establishment of individual risk calculation would dramatically help clinicians in determining which subjects would represent optimum candidates for early interventions.

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